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CHEMICAL ELIMINATION OF SAPROPHYTES DURING LABORATORY GERMINATION OF SEED PEAS[1]

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INTRODUCTION

The appraisal of the viability of a seed stock may be seriously handicapped by the presence of fungi and bacteria. Undeniably it is advantageous to determine the percentage of seeds actually infected by, or merely contaminated with, micro-organisms, for whether they be pathogens or saprophytes they depreciate the planting value of the seed. But it is essential in determinations of live seeds, and especially in comparing vigor of seedlings, that the micro-organisms be confined to their points of origin.

Perhaps the most instructive method of germination consists in placing the seeds widely apart on flat towels. The growth of the fungi and bacteria can be witnessed, yet at the same time the clean seeds are not contaminated by their neighbors during the germinative period. For the evaluation of viability, vigor, and associated micro-organisms this would be very exact. A spatial method of quarantine or isolation cannot be continuously practiced in routine germination work since sufficient space is not available. To maintain a high relative humidity and to conserve room in the germinator pea seeds (*Pisum sativum* L.) must be placed relatively close together on paper towels which are folded in loose rolls. Under these conditions fungi associated with a few seeds may spread over a part or all of a roll either by contact with adjacent seeds or seedlings or by growth of mycelium through the towel. Bacteria not only decompose the infected cotyledons, but also destroy the sprouts, and may weaken or kill surrounding normally strong seedlings. Decomposed radicles or plumules frequently lead to uncertain or incorrect readings, since it is often impossible to decide whether the growth of small rotted seedlings was naturally slow or was inhibited by saprophytes.

The mere presence of profuse mycelial growths, as illustrated by Koehler and Holbert (*12*)[3] for corn (*Zea mays* L.), is undesirable, even though no necrotic injury occurs. Manual handling of the tests is rendered unpleasant, and unless the obscuring fungus masses are removed the examinations are incomplete and the results uncertain. Freshly harvested mature seed stocks rarely prove troublesome. The small-seeded varieties of garden peas and all field peas usually harbor fewer fungus and bacterial associates than do the large-seeded varieties. Carry-over or storage stocks present more difficulty, roughly in propor-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 422.

tion to the percentage of dead or decadent seeds. Mixed lots or stocks resulting from indiscriminate blending of peas from returned packets or other small containers are commonly carriers of storage molds.

Tests in soil, while preferred by some laboratories on the basis of similarity to actual field conditions, are not without serious disadvantages. Unless the soil has a low inoculum potential the germination results may be an indication or measure of the soil condition rather than of the seed vitality. Moreover, examinations of the seeds for saprophytes on pathogens must be foregone.

On the thesis that chemicals which protect seeds in the soil should prove protective in germinator tests, the authors made a study of a number of dusts and dips to determine which would prove most effective in eliminating saprophytes during the germination of seed peas in the laboratory. The results of this study are reported in the present paper.

REVIEW OF LITERATURE

Observations of samples of germinating barley, cabbage, corn, and peas that had been treated for field planting strongly indicated that several materials were effective but more toxic in towels than in the soil. Horsfall and his coworkers (*7*) in their studies with seeds treated with cuprous oxide showed that injury occurs in sandy soil and clay subsoil.

Porter (*13*) reported that a dust compounded from New Ceresan (5-percent ethyl mercury phosphate) and talc was very valuable in his germination studies. Treated and nontreated samples of cereals germinating in the seed laboratory of the New York State Station showed that, on these seeds at least, the organic mercurials, and, to a lesser extent, certain copper compounds prevented the development of *Alternaria* spp., *Rhizopus nigricans* and *Penicillum* spp. The internally-borne *Helminthosporium* spp. of barley seed were not controlled by several copper compounds but were controlled by organic mercurials (*4*). Christensen and Stakman (*2*) stated that Ceresan (2-percent ethyl mercury chloride) effected practical control of seedling blights of barley. Bucha (*1*) suggested the laboratory possibilities of Ceresan by emphasizing its value in obtaining clean accurate tests of cottonseed. Hofer and Hamilton (*6*) in surface sterilizing legume seeds found an 0.006-percent concentration of ethyl mercury phosphate very effective.

EXPERIMENTAL MATERIALS AND METHODS

No attempt was made to secure highly contaminated lots of pea seed. The samples submitted for routine germination studies or those collected by inspectors from open stocks were utilized whenever possible. These varied from the finest current stock offerings of western seed growers to the blended contents of commission-box packets, or in other words, from very clean to severely infested seed. The germination of an untreated portion, usually 100 seeds or more, was compared with that of one or more treated portions depending on the size of the sample available. Seedling emergence from greenhouse soil was rarely used as an indication of the reliability of chemical protection. All laboratory portions were germinated at 20° C. in moistened paper towels, 10 by 14 inches, folded into loose rolls of about 1½-inch diameter. The rolls were unfolded after 6 days for examination of the seedlings. Seed producing either strong, normal sprouts and weak sprouts which appeared to have been developing normally prior to

microfloral attack or to chemical injury were counted as germinated. The degree of contamination and rotting was observed and usually recorded as the percentage of seeds injured by, or in contact with, any pathogen or saprophyte. The general condition of the treated lots was compared with that of the control. Particular attention was paid to acceleration of growth, length of plumules and radicles, development of secondary and vesicle roots, and presence of chemically induced abnormal seedlings. As a statistical basis for comparison of development, the radicles and plumules of the seedlings were measured in some experiments while in many others the entire portion (100 seeds and seedlings) was weighed.

Materials known to be effective in control of smut, seedling rots, damping-off, storage decay, and other diseases were requested from the manufacturers. These were applied initially as recommended by plant pathologists or by the manufacturers, but several were later altered to change the rate of adherence. The following chemicals were used:[4]

Mercury compounds:

Volatile: 2-percent ethyl mercury chloride (Ceresan), 1-percent ethyl mercury phosphate (New Semesan Jr.), 5-percent ethyl mercury phospate (New Ceresan), and 30-percent hydroxylmercurichlorophenol (Semesan).

Nonvolatile: 8-percent ethyl mercury cyanamide (Barbak C), mercuric chloride, mercurous chloride, a 3-percent metallic mercury dust (Merko), 2-percent ethanol mercury chloride (Sanoseed), and phenyl mercury nitrate.

Copper compounds:

Very soluble: Basic and monohydrated copper sulphate.

Slightly soluble—copper carbonate (25-, 50-, and 52-percent copper content), copper hydroxide, copper oxalate, copper oxychloride, copper phosphate, copper stearate, cuprous oxide, and a dusting copper of unknown composition.

Other materials:

Zinc: Zinc chromate, zinc oxide, zinc stearate, and mixed zinc compounds (Vasco 4).

Aldehydes: Formaldehyde dusts (Ansul, Corona, and Smuttox), and methyl aldehyde plus copper and mercury (Grainaide).

Miscellaneous: Ammonium sulphate, borax, copper-lime-arsenite, ferrous sulphate, potassium dichromate, potassium iodide, and dusting sulphur.

The chemicals were applied as dusts by agitating the seeds with the chemical in an Erlenmeyer flask or a fruit jar. The excess chemical was removed by shaking the seeds in a tea strainer. The seeds were either immediately placed on towels, planted in the soil, or held in tight paraffin containers for 48 to 72 hours before the beginning of germination. A wet or dip method proved easier in practice in that the seeds were simply dropped into a beaker of the suspension or solution of the test chemical, collected by pouring the liquid and seeds into a tea strainer, and immediately spaced on the towels. This method was altered infrequently; the dry seeds were placed in a tea strainer, and liquid was poured over them once or twice. Usually the working portions of both the dusts and dips were used continuously until exhausted. The chemically treated samples were always compared with control samples and germinated under identical conditions. Because of the limited size of the seed stocks, machine treatments with a determined weight and volume of chemical were impractical. However, numerous samples were drawn from cuprous oxide- and graphite-dusted bulks. The microflora and the vigor of the seedlings were regularly noted and compared with untreated seeds of the same variety.

[4] The cooperation of the manufacturers in supplying these chemicals is gratefully acknowledged.

EXPERIMENTAL RESULTS

DUST TREATMENTS

The majority of the chemicals employed in this study were processed for use as dusts. In treating large quantities of seeds dry applications are obviously preferable to dip or soak treatments. In order to secure results which would be comparable to control of micro-organisms on a commercial basis the first experiments in the laboratory were performed with commercially available dusts.

The recommended procedure of agitating the seeds and chemical for a period of 5 to 10 minutes was not practical in the routine laboratory studies. Moreover, certain preliminary trials with shaking periods of 30 seconds to 10 minutes showed that fungus control was no better for the 5-to-10-minute than for the 30-to-60-second treating period. Dilution of the manufactured product by an inert material was necessary with several chemicals in order to reduce the injury to the pea sprouts. Usually, however, the maximum amount of dust adhering to the dry seeds closely approximated the recommended dosage of 2 to 2½ ounces per bushel of seed, and the addition of a diluent to the chemical was not required.

Ethyl Mercury Phosphate

Since routine germination tests with treated cereals had repeatedly proven that certain mercurials would prevent the discoloration of seeds by *Alternaria* sp. and *Helminthosporium* spp., these materials were studied extensively as treatments for the larger agricultural seeds. Porter (*13*) suggested the use of a 1-percent ethyl mercury phosphate dust for protecting corn sprouts. The dust, made by blending one part of New Ceresan with four parts of French talc, is equivalent to the commercially compounded product, New Semesan Jr., which is widely applied to sweet- and field-corn seed. Porter found that the use of diluted New Ceresan expedited the reading of germinated corn because both saprophytes and pathogenes were eliminated. Cuprous oxide did not prove satisfactory.

Koehler (*10*) reported that the recommended dosage of New Ceresan (one-half ounce per bushel) for oats was probably heavier than actually required for smut control. In fact, a ⅜-ounce dosage appeared effective, even through the seed was planted before the mercury could volatilize. Later he (*11*) observed that cuprous oxide protected crown-injured corn seed better than did the mercury compounds, but in the control of seed-borne organisms, the opposite was true.

Under the assumption that approximately 2¼ ounces of talc would adhere to a bushel of pea seeds, a mixture of one part of New Ceresan with five parts of talc was prepared in order to obtain a per-bushel dosage equivalent to three-eighths of an ounce of the undiluted chemical. A mixture containing 0.833 percent of ethyl mercury phosphate was used in several thousand germination tests with results that were uniformly satisfying. When seeds are shaken with the dust in a flask the dosage per bushel naturally varies with the size, moisture content, surface characters of the seed, and to some extent with the period of agitation. Onion, radish, and certain flower seeds were injured occasionally, but in general they tolerated comparatively large dosages. Pea seeds, on the other hand, having a low surface volume ratio, were not heavily treated. Presumably the 0.833-percent

ethyl mercury phosphate dust unless given time to volatilize might not control fungi, but, as shown in table 1, this dust actually was very effective, allowing no, or seldom a trace of, saprophytes to develop.

Bentonite clay was also used as the inert component in diluting New Ceresan. It appeared to be but little different from talc in adherence.

The content of ethyl mercury phosphate in the dust was further reduced. A mixture containing only 0.455 percent of this organic mercurial proved adequate for practical control. The fungicidal value of this dust was only slightly lower than that of the 1 to 5 mixture while the seedling weight was increased by its use (table 1).

TABLE 1.—*Influence of dilution of New Ceresan dust, or of storage of the treated seeds, on germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

Type or variety of seed and dust used	Period of storage	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests (ratio [4])
			Rhizopus nigricans	*Penicillium* sp.	*Dematium pullulans*	Bacteria		
New-crop bulks:	*Hours*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
New Ceresan 1, talc 5 [1]	0	92.7	0	0.1	0	0.5	40.1	4/43
Check		91.0	5.1	2.1	11.3	2.4	36.8	0/43
Thomas Laxton:								
New Ceresan 1, talc 5 [1]	0	96.8	.1	.4	0	4.7	37.8	1/20
Check		90.7	11.9	3.5	16.7	7.9	35.7	0/20
Stored bulks:								
New Ceresan 1, talc 5 [1]	0	91.3	.1	.1	0	1.5	46.7	1/26
New Ceresan 1, talc 10 [2]	0	91.5	.3	.1	.3	1.5	48.0	0/26
Check		84.6	30.3	2.4	21.5	8.4	42.4	0/26
Alderman:								
New Ceresan 1, talc 5 [1]	0	86.6	.6	.1	.1	6.8	41.3	0/28
Do.[1]	[3] 72	85.0	.6	.2	0	6.7	40.2	1/28
Check		78.7	42.3	7.9	26.4	16.0	36.9	0/28
Thomas Laxton:								
New Ceresan 1, talc 5 [1]	0	80.9	.6	.5	0	8.8	39.8	0/27
Do.[1]	[3] 48	81.2	.9	1.3	0	9.6	39.4	0/27
Check		72.3	52.2	13.9	11.8	21.7	34.2	0/27

[1] A blended mixture of 1 volume of New Ceresan with 5 volumes of talc.
[2] A blended mixture of 1 volume of New Ceresan with 10 volumes of talc.
[3] Seeds were stored in tight paraffin containers immediately after treatment.
[4] The numerator is the number of 100-seed tests containing chemically injured sprouts, the denominator is the total number of tests.

DELAYED INITIATION OF GERMINATION OF SEEDS TREATED WITH ETHYL MERCURY PHOSPHATE

Since ethyl mercury phosphate is volatile, the fungicidal action of New Ceresan depends not only upon the dosage used but also upon the time elapsed between treatment and germination. Koehler and Holbert (*12*), Porter and Layton (*14*), and Johnson et al. (*8*) have recommended that smut-infected cereals be treated 1 or 2 days prior to seeding. Probably very few phytopathologists have observed the daily growth of sprouts from treated seeds. Hence they have little knowledge of whether or not a storage period is required to render New Ceresan effective against *Rhizopus nigricans*, *Penicillium* spp., *Alternaria* spp., *Helminthosporium* spp., and other fungi on cereals. Bucha (*1*) commented on the remarkable fungicidal action of Ceresan against fungi commonly contaminating cotton seeds. Apparently a storage period was not necessary. Koehler (10) applied various dosages of New Ceresan to oats and stored the seed from 2 hours to 72 days before seeding. Smut elimination was effected by a ⅛-ounce-per-bushel treat-

ment in a 72-day storage, by ¼-ounce treatment in a 5-day storage, or by ½-ounce treatment in a 2-hour storage.

Porter (*13*) recommended storage of the treated seeds for 24 hours to avoid mercury injury. No mention was made of enhancing the fungicidal action. In order to determine the value of delaying the initiation of germination 55 samples of new-crop western pea seed were treated with New Ceresan by different methods in the New York State Station laboratory. The samples were divided into two portions: One portion was agitated in a flask containing the 1 part New Ceresan to 5 parts talc dust while the other was left as a control. The dusted seeds were divided into 2 parts, one part was placed to germinate immediately, the other stored for 48 or 72 hours before germination. Each lot was removed in exactly 144 hours and the percentage of germinated seeds recorded. The results (table 1) indicate that there is no advantage in delaying germination and also that a short storage period is not conducive to mercury injury.

Other Volatile Mercurials

The proprietary dusts, Ceresan, New Semesan Jr., and Semesan, which also contain volatile mercurials, were found useful as substitutes for New Ceresan. They were applied in the original manufactured forms without reduction with an inert dust. Dilutions to avoid mercury poisoning of the seedlings apparently were not necessary. Injury was noted in a few trials, but undoubtedly it was caused by failure to remove all of the excess dust. These materials, when present in amounts greater than those normally adhering to dry pea seeds, were toxic and caused abnormal growth of the seedlings.

Table 2.—*Influence of certain proprietary dusts and mercury compounds on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the size and green weight of, and chemical injury to, the sprouts*

Dust applied to seed	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests (ratio[2])
		Rhizopus nigricans	*Penicillium spp.*	*Dematium pullulans*	Bacteria				
PROPRIETARY DUSTS CONTAINING VOLATILE CHEMICALS									
Ceresan:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
Treated	90.4	0.4	0.2	0.1	7.0	2.7	2.7	41.9	2/63
Check	79.7	32.8	1.6	31.4	16.3	2.2	1.8	26.5	0/63
New Semesan Jr.:									
Treated	85.8	0	.1	0	.6	.....	.....	42.7	0/10
Check	81.9	17.3	2.4	18.5	6.9	.....	.....	40.1	0/10
Semesan:									
Treated	95.5	0	6.5	0	.5	2.6	2.5	70.5	0/8
Check	90.0	3.2	1.4	11.3	1.5	2.3	2.3	66.1	0/8
PROPRIETARY DUSTS CONTAINING NONVOLATILE CHEMICALS									
Barbak C:									
Treated	94.0	2.2	1.8	0	3.2	.....	.....	43.0	3/10
Check	90.2	28.2	9.8	25.0	6.0	.....	.....	45.5	3/10
Merko:									
Treated	90.8	1.4	.4	0	2.8	1.7	2.7	45.1	1/60
Check	85.7	28.0	3.9	48.0	8.6	1.5	1.6	39.2	0/60
Sanoseed:									
Treated	89.8	5.0	2.0	0	3.7	.....	.....	40.8	8/40
Check	91.6	14.4	5.4	17.8	6.6	.....	.....	40.3	0/40

TABLE 2.—*Influence of certain proprietary dusts and mercury compounds on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the size and green weight of, and chemical injury to, the sprouts*—Continued

DILUTIONS OF NONVOLATILE CHEMICALS

Dust applied to seed	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests (ratio)
		Rhizopus nigricans	*Penicillium spp.*	*Dematium pullulans*	Bacteria				
Mercuric chloride:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
17 percent [1]	84.3	0	0	0	0			29.2	1/10
Check	85.5	39.2	.3	95.5	4.2			30.5	0/10
Mercurous chloride:									
Undiluted	92.0	.5	.6	0	1.6			33.5	6/10
Check	86.8	13.6	3.2	4.8	5.8			36.4	0/10
Phenyl mercury nitrate:									
25 percent [1]	93.1	.1	.1	.3	1.5			38.3	7/25
Check	90.9	9.6	6.3	35.4	4.3			39.5	0/25
10 percent [1]	94.4	.3	.7	0	1.5			49.2	0/12
Check	93.9	9.0	3.8	25.0	4.1			48.6	0/12
5 percent [1]	94.9	.1	.3	0	2.1			48.2	0/16
Check	92.8	1.2	1.8	9.8	3.6			47.8	0/16

[1] Diluted with bentonite clay, the chemical is expressed in percent by volume.
[2] See footnote 4, table 1.

As shown in table 2, Ceresan effectively reduced fungus contaminations. This dust is outstanding for its uniform control of fungus saprophytes irrespective of the variety or age of the seed, or of the species of fungus. Bacteria were more tolerant of this chemical (ethyl mercury chloride) than were fungi, and complete elimination of bacteria was never observed. Ceresan did, however, localize the bacteria to the dead seeds or points of infestation and usually inhibited their rapid decay. The slimy condition, so objectionable in handling untreated tests, was practically unknown in Ceresan-treated rolls of peas.

Radicles in the Ceresan-treated tests were consistently longer than those in the untreated tests largely because Ceresan prevented fungi and bacteria from destroying them. In several lots of stored seed the average lengths of radicles in the untreated tests were only one-half the length of those in the treated rolls. The differences between radicle lengths of treated and untreated seeds were usually less in freshly harvested than in stored peas of the same varieties. Comparative radicle lengths for 23 uncontaminated samples were 2.3 inches for the untreated, and 2.9 inches for the Ceresan-treated. In tests of stored seed Ceresan often increased the length of radicles by 1.0 inch.

The plumules were never seriously injured by associated saprophytes, and consequently Ceresan effected only slight increases in their lengths.

Comparative green weights of treated and untreated seeds also strongly indicated that Ceresan increased the growth of pea sprouts. In 11 highly contaminated lots the average weight of sprouts from 100 untreated seeds was 26.8 gm., from treated seeds 60.6 gm. Similar weights for 33 noncontaminated lots were 39.9 gm. for the untreated and 44.3 gm. for the treated.

New Semesan Jr. and Semesan were used with a limited number of varieties of pea. They generally decreased the percentage of con-

taminated seeds and usually increased the percentage of apparently normal seedlings. Gains in weight of sprouts were not so consistently secured by these mercurials as with Ceresan. Uniform dosages were easily obtained by the excess dust-hand-agitation method since both New Semesan Jr. and Semesan adhered well, and the excess dusts were easily removed.

Nonvolatile Proprietary Dusts

The only serious disadvantage in the application of the highly effective volatile dusts in the seed laboratory is the danger of inhaling the poisonous gases. For this reason certain nonvolatile dusts were used extensively. When a choice was permissible, the laboratory assistants invariably selected one of the latter materials. The data in table 2 were averaged from at least 10 varieties used as test material for each dust.

From a consideration of germination, fungus elimination, development of sprouts, and lack of injury, Merko was found to be very satisfactory for laboratory work. The other materials were equally suitable in several respects, but for excess dust treatments the percentages of the active chemicals in each dust apparently were too high.

Dilutions of Nonvolatile Pure Chemicals

A dust composed of one part of mercuric chloride and five parts of clay by volume had a high fungicidal-phytocidal ratio. As shown in table 2 highly contaminated peas were rendered entirely free of both fungi and bacteria by treatment with the diluted mercuric chloride dust. Chemical injury was noted in only one test, and that was probably the result of improper mixing of the materials. Later experiments showed that as a laboratory fungicide mercuric chloride compares favorably with New Ceresan. Dusts containing 17 percent of either material consistently eliminated fungi although mercuric chloride seemed not to accelerate growth or to effect increases in green weight. Even when diluted with 10 times its bulk of clay, mercuric chloride decreased both fungi and bacteria to a degree that permitted accurate and rapid counting of normal sprouts. Germinants developing in contact with mercurous chloride may exhibit all the typical characteristics of mercury poisoning. In spite of the low solubility of mercurous chloride in water sufficient amounts of the chemical will adhere to pea seeds to cause marked retardation of growth. When carefully applied to dry seeds and with the excess entirely removed only slight injury was observed. On several occasions the chemical adhering to moist pea seeds completely ruined the rolls of germinants. On the other hand dilution of mercurous chloride with equal volumes of an inert dust entirely prevented phytotoxicity, but contaminants were not eliminated. In ease of application, freedom from volatilized gas, and absence of discoloration of working surfaces the mercurous compound compares favorably with mercuric chloride. Since the latter can be diluted with five times its volume of an inexpensive inert material it is preferable to the more expensive mercurous chloride. The two compounds are compared in table 2.

Phenyl mercury nitrate although very insoluble in water proved to be an efficient seed protectant. It was easily compounded with either talc or clay. The optimum dilution varied somewhat with the variety of pea seed and evidence of infestation but a mixture of one part of

chemical to nine parts of diluent proved satisfactory in several experiments. Compared to either of the ethyl mercury compounds phenyl mercury nitrate would be exorbitantly expensive even for laboratory disinfestation.

Diluted and Undiluted Cuprous Oxide

The first studies with chemical protectants were undertaken to adapt the commonly used dust for peas, cuprous oxide, to laboratory technique. Many varieties of peas containing varying percentages of saprophytes were treated. Applications of the undiluted chemical by the excess dust method usually resulted in severe injury to the sprouts. Considerably more than the recommended dosage of 2 ounces of dust per bushel of seed actually adhered to the peas. Weight increases of seeds indicated that the 4-ounce rate was often obtained. Since the extremely small amount of chemical required for 100 seeds was difficult to control because of loss on the surface of the treating flask the excess dust method was modified by adding a diluent to the pure cuprous oxide. The per-bushel rate was easily reduced to 2 ounces or as much less as was desired. This method was followed in the experiments the results of which are summarized in table 3.

Table 3.—*Influence of cuprous oxide, either undiluted or diluted with clay, on the germination and on the saprophytic and bacterial contaminants of pea seeds, and the size and green weight of and chemical injury to the sprouts*

Type of peas and dust applied	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests, ratio [3]
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria				
Small-seeded:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
Cuprous oxide	89.0	0.4	0.8	.....	3.9	1.8	1.2	45.0	20/20
Cuprous oxide, clay [1]	88.3	1.5	.9	.....	3.1	1.8	1.6	43.7	14/20
Check	90.3	7.5	1.4	.....	3.1	2.2	3.1	51.3	0/20
Large-seeded:									
Cuprous oxide	88.7	9.9	1.2	.....	7.2	1.1	1.3	41.0	20/30
Cuprous oxide, clay [1]	88.3	14.8	2.3	.....	8.8	1.3	1.9	44.8	22/30
Check	87.2	30.3	6.1	.....	12.7	1.3	2.3	48.3	0/30
Year-old seed:									
Cuprous oxide	90.5	4.8	.9	0	18.4	1.7	1.1	50.1	75/79
Check	75.4	41.2	2.3	0	29.5	1.9	2.4	44.8	0/79
Canning varieties:									
Cuprous oxide	86.6	.1	0	0	1.4	1.8	1.2	.....	8/8
Cuprous oxide, clay [1]	85.5	.5	.6	0	.5	1.8	1.6	.....	6/8
Do [2]	85.3	1.5	.3	0	1.5	2.0	2.4	.....	4/8
Check	85.0	3.5	1.7	11.2	1.4	2.2	3.1	.....	0/8

[1] Equal volumes of cuprous oxide and bentonite clay.
[2] 1 volume of cuprous oxide to 4 volumes of bentonite clay.
[3] See footnote 4, table 1.

Cuprous oxide was reduced by one or four times its volume of bentonite clay and used in comparison with the unreduced cuprous oxide. The 50-percent cuprous oxide dust gave practical control of molds and bacteria. Seedling injury was less severe in most trials, but in general a decrease in injured radicles was accompanied by a slight increase in molds. The 20-percent cuprous oxide in clay dust further reduced the seedling injury but unfortunately caused a sacrifice in fungus control. Ceresan and New Ceresan usually effected better control than unreduced cuprous oxide and always caused less injury than did 20-percent cuprous oxide.

Copper Stearate and Combinations of It with Other Chemicals

It is difficult to remove cuprous oxide from laboratory benches and tables, and the red spots remaining are objectionable. Furthermore, its phytocidal action overshadows its value as a laboratory fungicide. Copper stearate is preferable in respect to color, adhesiveness to seeds, and ease of removal from working surfaces. Its substitution for cuprous oxide could be justified on these characteristics alone.

TABLE 4.—*Influence of copper stearate used alone and in combination with other chemicals on the germination and fungus contaminants of pea seeds, and on the size of and chemical injury to the sprouts, with comparative data on the use of other seed disinfectants*

Test Nos. and chemical applied as dust	Germination	Fungus contaminated seeds	Length of plumules		Length of radicles		Chemically injured tests ratio [2]
			Average	Maximum	Average	Maximum	
1 to 9:	*Percent*	*Percent*	*Inches*	*Inches*	*Inches*	*Inches*	
Copper stearate	94.9	0.9	1.5	2.3	1.9	3.1	3/9
Copper sulphate	87.6	4.1	1.5	2.2	1.3	2.6	7/9
Cuprous oxide	91.8	1.1	1.5	2.2	.8	1.8	9/9
Merko	95.4	2.0	1.8	2.9	2.2	3.6	0/0
Zinc oxide	98.7	2.2	1.3	2.1	1.0	2.2	8/9
Check	29.1	59.5	1.1	2.0	1.4	2.8	0/9
10 to 17:							
Copper stearate	94.4	0	1.6	2.5	1.5	2.7	2/8
Copper sulphate	91.4	0	1.6	2.5	1.2	2.5	5/8
Cuprous oxide	92.5	.5	1.4	2.3	.8	1.7	6/8
Merko	92.7	0	1.8	2.8	2.4	3.4	1/8
Zinc oxide	90.2	.6	1.3	2.2	.9	1.8	7/8
Check	87.3	43.3	1.4	2.3	1.2	2.0	0/8
18 to 24:							
Copper carbonate	66.2	16.1	.8	1.3	.8	1.6	5/7
Copper stearate	88.8	1.8	1.2	1.9	1.5	2.5	1/7
Copper sulphate	71.8	12.1	1.1	1.9	1.0	1.7	6/7
Copper carbonate-copper stearate [1]	64.2	4.9	1.0	2.0	.7	1.7	7/7
Copper sulphate-copper stearate [1]	34.5	1.2	.5	1.1	.5	1.2	7/7
Check	55.2	83.5	.8	1.7	1.0	2.3	0/7

[1] Equal volumes of each chemical.
[2] See footnote 4, table 1.

The data in table 4 show that copper stearate not only is less toxic to pea seedlings than are certain other copper compounds but also it is a better fungicide. This does not accord with various reports of soil tests in which copper stearate appeared to be almost worthless as a seed protectant. The results of the present study, however, indicate that the dosage could be reduced below that of normal adherence without a sacrifice of fungus elimination and seedling protection.

Dusting pea seeds with copper stearate rarely caused an abnormal development of the radicles. A few injured sprouts were found in several tests of seed treated with the undiluted dust. However, the ratio of tests in which sprouts were injured to the total number of tests was never high (tables 4 and 5). Furthermore, the radicles even when injured were not so short and malformed as were those developing in the cuprous oxide-treated rolls. Severe burning and killing of the root tips were not observed.

The plumules from copper stearate-dusted seed were always longer than those from contaminated and untreated peas. In comparison with other copper compounds copper stearate allowed a more normal

development of plumules as well as radicles. An actual chemical stimulation probably did not result. Mercury compounds, as represented by Merko in table 4, invariably effected a greater apparent stimulation on either contaminated or fungus-free lots of peas.

TABLE 5.—*Influence of copper stearate dust, copper stearate amended with clay, and New Ceresan, and with both clay and New Ceresan on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

Variety of pea seed and chemical applied as dust	Germination	Seeds or sprouts contaminated with—			Green weight of sprouts	Chemically injured tests (ratio [7])
		Rhizopus nigricans	*Dematium pullulans*	Bacteria		
Alderman and World's Record:	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
Copper stearate-New Ceresan [1]	65.5	0.5	0	32.3	48.4	11/11
Check	57.5	49.6	11.3	24.1	36.8	0/11
Alaska and Gradus:						
Copper stearate-New Ceresan [1]	89.3	.2	0	20.4	38.9	19/19
Check	83.8	33.8	27.6	26.3	54.9	0/19
Perfection:						
Copper stearate	94.6	.3	8.3	.4	43.1	0/20
Copper stearate-clay [2]	95.1	4.8	5.3	.6	45.8	0/20
Copper stearate-New Ceresan [3]	94.8	0	0	0	38.3	16/20
Check	94.5	6.5	79.6	1.7	44.1	0/20
Thomas Laxton:						
Copper stearate-New Ceresan [4]	91.4	.1	0	1.1	40.2	14/14
Check	85.6	79.5	0	8.9	37.1	0/14
Rogers Chief:						
Copper stearate-New Ceresan [4]	95.1	0	0	.2	37.8	7/15
Check	93.5	3.8	83.3	1.0	39.0	0/15
Wisconsin Early Sweet:						
Copper stearate-New Ceresan [4]	97.0	0	0	.2	33.2	4/10
New Ceresan-clay [5]	98.3	.2	0	.4	33.0	2/10
Check	99.2	11.8	.4	1.0	34.3	0/10
Rogers Chief:						
Copper stearate-clay-New Ceresan [6]	95.4	0	0	0	40.6	0/18
Check	96.0	0	100.0	0	42.8	0/18
Surprise:						
Copper stearate-clay-New Ceresan [6]	99.0	0	0	.3	35.5	3/10
Check	97.1	16.2	0	.8	33.2	0/10
Climax:						
Copper stearate-clay-New Ceresan [6]	93.2	0	0	2.8	23.4	2/15
Check	89.4	17.8	0	7.2	22.0	0/15
Alaska:						
Copper stearate-clay-New Ceresan [6]	96.3	0	0	.7	30.3	0/15
Check	95.3	2.3	0	1.7	29.0	0/15

[1] A blended mixture of 17 percent of New Ceresan and 83 of copper stearate, by volume.
[2] Equal volumes of copper stearate and bentonite clay.
[3] A blended mixture of 9 percent of New Ceresan and 91 of copper stearate, by volume.
[4] A blended mixture of 5 percent of New Ceresan and 95 of copper stearate, by volume.
[5] A blended mixture of 17 percent of New Ceresan and 83 of bentonite clay by volume.
[6] A blended mixture of 80 percent of copper stearate, 16 of bentonite clay and 4 of New Ceresan by volume.
[7] See footnote 4, table 1.

Since monohydrated copper sulphate is toxic to pea seedlings, it was blended with an equal volume of copper stearate in an attempt to reduce seedling injury or at least to effect decreases in fungus contaminations. The seeds were treated by the hand-agitation method, and the excess dust was removed entirely. The mixture appeared to adhere almost as well as did copper stearate. By measuring the exhaustion of material from the treating flasks it was found that the amount of copper sulphate adhering to the seeds was not decreased by the presence of copper stearate. In fact, the greater adhesiveness of the latter entirely compensated for its diluting effect. Naturally, then, as shown in table 4, the toxicity of this mixture exceeded that

of either chemical alone, while the percentage of contaminated sprouts was reduced slightly.

A copper carbonate—copper stearate mixture was also compared with the pure chemicals. The seed coverage was slightly greater for the mixture than for copper carbonate alone. The amount of this chemical which adhered to each seed was decreased by the diluent, copper stearate. The germination records indicated that with respect to fungus contaminations and length of plumules and radicles the mixture was inferior to copper stearate alone and only slightly better than copper carbonate.

Copper stearate and New Ceresan were compounded to reduce the toxicity of copper and to prevent the diffusion of volatilized ethyl mercury phosphate. Copper stearate also was diluted with clay by mixing equal bulks of each together.

The first mixtures of copper stearate and New Ceresan compounded for laboratory treatments contained 17 percent of the latter material. The developing pea seedlings were definitely injured, although the apparent germination percentage was often increased. When the seeds were from storage lots the reduction in fungus contaminations due to chemical control usually compensated for the phytocidal effect. The green weight of the sprouts detached from treated seeds was greater than that of the sprouts from untreated peas in a few series of tests, but, as shown in table 5, chemical injury usually caused a marked reduction in green weight.

The 9-percent New Ceresan–91-percent copper stearate mixture also was injurious to the sprouts. When the former component did not exceed 5 percent of the total volume of dust, only a few sprouts appeared to be abnormal. It was necessary, however, to add clay or another diluent to the mixture of New Ceresan and copper stearate to obtain a dust as efficient and safe as diluted New Ceresan alone. The mixture of 80 percent of copper stearate, 16 percent of diluent, and 4 percent of New Ceresan by volume was applied to many large agricultural seeds. Fungus contaminations never exceeded 1.0 percent. The results (table 5) indicated that the copper stearate-clay-New Ceresan mixture is a very efficient, practically nontoxic dust. It is not so disagreeable to use in the laboratory as is a New Ceresan-talc mixture containing 0.83 percent of ethyl mercuric phosphate, is a better fungicide than a copper stearate-clay mixture, and is less toxic to pea seedlings than is undiluted copper stearate. It was tested experimentally on some 30 varieties of peas, with only slight varietal differences.

Other Copper Compounds

A number of copper compounds, especially those of low solubility in water have caused varying amounts of injury. The influence of a few of these on pea seeds is shown in table 6. The relative value of the materials is indicated by comparison with the New Ceresan and clay mixture. Since the usefulness of a dust depends primarily upon its efficiency in eliminating or localizing saprophytes, it appears that most of the copper compounds cannot be recommended. Good control was secured in some lots of seeds but not in others. By and large, however, several of the more insoluble compounds, the carbonate, hydroxide, and oxychloride in particular reduced molds sufficiently to permit accurate appraisal of the results of the germination tests.

TABLE 6.—*Influence of certain copper dusts on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

Variety or type of pea seed and chemical compound applied as dust	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests ratio [3]
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria		
Canning bulks:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
Copper carbonate	95.3	1.0	0	0	0	36.6	10/10
Copper hydroxide	95.5	.7	0	0	0	36.6	10/10
Copper oxalate	97.0	5.0	.7	0	.5	41.2	8/10
Copper oxychloride	95.7	.3	.0	0	.3	39.3	8/10
Copper sulphate	94.0	2.0	.7	0	.7	37.7	10/10
Cuprous oxide, clay [1]	95.0	.7	.3	0	.6	38.8	10/10
New Ceresan, clay [2]	97.7	0	0	0	.1	40.2	0/10
Check	94.3	11.7	.7	32.3	2.1	41.2	0/10
Commission packets:							
Dusting copper	63.7	59.5	2.5	0	13.2	38.0	9/10
Check	61.5	81.2	2.0	0	23.2	39.5	0/10
Canning peas, 3 varieties:							
Copper oxalate	85.7	15.3	1.3	0	6.7	50.7	8/10
Check	80.0	71.7	1.0	0	17.3	49.3	0/10
Canning peas, 7 varieties:							
Copper phosphate	79.6	74.2	5.2	0	18.0	45.2	0/15
Check	77.1	78.6	3.7	0	19.3	44.3	0/15

[1] A mixture of equal bulks of cuprous oxide and bentonite clay.
[2] A mixture of 1 part of New Ceresan and 5 parts of bentonite clay.
[3] See footnote 4, table 1.

ZINC COMPOUNDS AND OTHER CHEMICALS NOT COMMONLY USED AS SEED PROTECTANTS

Various zinc compounds, sulphur, copper-lime-arsenite dusts, and aldehydes in inert diluents were tested on several varieties of peas. In comparison with the copper and mercury compounds the former group was not impressive. Zinc oxide was not a good fungicide even when the dosage applied was injurious to the sprouts. Vasco 4, which contains the oxide and other compounds of zinc was never so effective as the oxide alone, nor did it effect as definite a retardation of radicle elongation. Further reduction of the available zinc by mixing graphite with Vasco 4 resulted in a material with only slight fungicidal properties. Zinc chromate and zinc stearate were superior to zinc oxide chiefly because they did not injure the sprouts. Table 7 presents an accurate comparison of these materials.

Zinc oxide and, to a lesser extent, other zinc compounds were easily adapted to laboratory usage. They adhered readily to every variety of pea seed. In removal of the excess dust continued sieving seemed to have no effect on the seed coverage so that a uniform dosage was obtained in all trials. The laboratory technicians often expressed a preference for zinc oxide or Vasco 4 largely because these materials were not volatile. That they did not discolor the table and germinator surfaces was the important factor in their selection over other nonvolatile protectants.

Sulphur controlled *Rhizopus nigricans* in only a few lots of peas. Mycelium usually developed from dead seeds regardless of the sulphur coating. When this dust was applied in excess of a normal coverage the percentage of contaminated seeds was decreased. Such a procedure could not be generally followed, however, because it was difficult to determine the amount which would be fungicidal but not phytocidal.

TABLE 7.—*Influence of certain fungicides on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

Variety or type of pea seed and material applied as dust	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests, (ratio [1])
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria		
ALDEHYDES							
Retail packets, 10 varieties:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
Corona Oat Dust	81.6	20.1	2.0	2.6	8.1	49.0	0/20
Check	76.9	21.9	4.1	34.5	10.3	47.7	0/20
Formacide	78.6	32.1	2.9	3.0	14.9	53.5	2/20
Check	73.1	43.9	4.3	7.5	18.0	48.9	0/20
Perfection and Horsford:							
Grainaide	74.3	63.2	1.0	0	15.8	47.3	1/25
Check	71.0	74.8	2.4	18.3	17.0	45.8	0/25
Telephone and Laxtonian:							
Smuttox	94.3	5.5	1.0	0	9.1	33.7	1/10
Check	90.6	11.7	3.9	16.8	11.3	36.2	0/10
COPPER-LIME-ARSENITE							
Garden varieties:							
No. 395	72.5	3.4	1.1	0	3.9	45.0	10/10
No. 397	64.5	9.3	.0	.0	31.1	42.5	10/10
Check	85.0	48.7	.5	1.0	11.9	56.1	0/10
Commission boxes, 10 varieties:							
No. 395	79.1	1.6	.6	0	3.9	34.0	15/15
Check	91.0	26.6	3.2	1.8	5.2	41.1	0/15
No. 397	50.6	15.3	10.1	0	29.3	31.7	16/16
Check	80.6	44.4	4.1	5.5	14.4	45.5	0/16
SULPHUR							
Canning varieties:							
Dusting sulphur	81.8	18.6	0	0	10.8	36.2	0/10
Check	89.4	22.0	1.0	0	6.2	35.6	0/10
Retail packets, 10 varieties:							
Dusting sulphur	85.0	15.3	1.7	0	8.2	40.9	1/10
Check	81.6	35.8	3.3	0	13.3	40.0	0/10
ZINC COMPOUNDS							
Thomas Laxton:							
Zinc chromate	92.0	17.9	2.0	0	4.0	.....	3/12
Check	76.0	48.2	0	0	16.5	.....	0/12
Wisconsin Early Sweet and Perfection:							
Zinc oxide	93.3	9.2	.5	6.5	1.9	54.7	16/20
Check	92.2	18.7	1.0	9.0	4.1	37.2	0/20
Garden varieties:							
Zinc stearate	85.7	10.4	3.0	.9	4.7	89.1	2/11
Check	81.8	43.5	9.6	13.3	11.7	88.3	0/11
Perfection and Surprise:							
Vasco 4	88.2	7.2	1.8	27.5	3.6	37.3	0/20
Check	85.8	16.2	3.6	33.8	6.5	37.4	0/20
New-crop seed:							
Vasco 4	96.0	15.0	2.4	50.0	1.9	48.5	3/20
Vasco 4 plus graphite	95.3	29.5	3.8	43.7	2.4	53.3	0/20
Check	94.7	25.0	5.6	51.8	3.6	51.9	0/20

[1] See footnote 4, table 1.

Several volatile materials recommended for control of stinking smut of wheat and oat smuts were included in this work. The dry pea seeds were treated as in former experiments by agitating them in a flask with the dust, followed by a removal of the excess material. The seeds were at once placed on damp towels which were folded into loose rolls. No attempt was made to confine the gases by storing

the seeds for a few hours or days as is customary with formaldehyde and methyl aldehyde dusts when employed in smut control. That the folded towels prevented rapid diffusion of the vapors is possible.

The results (table 7) indicate that the black-spot fungus (*Dematium pullulans*) is easily controlled. This is to be expected since it is confined to the surface of the seed coat and can be killed before the formaldehyde gas escapes. Naturally neither the formaldehyde dusts nor Grainaide could protect the clean seeds from contamination by *Rhizopus nigricans* or *Penicillium* spp. The fact that injury to the sprouts has only rarely been observed proves definitely that the vapors diffuse from the towels within a few hours.

The copper-lime-arsenite dusts,[5] valuable as foliage protectants and for control of stinking smut of wheat, were found to be good fungicides, but they severely injured the seedlings. They were used as compounded in Wisconsin for smut control or foliage dusts. A comparison of the results from the two dusts indicated that the toxicity could be reduced by the addition of an inert carrier. The increase in percentage of bacteria-contaminated seeds was attributable entirely to killing of the seeds.

Comparison of Several Dust Protectants

A number of ½-pound bulks of pea seed for sale in 1935 germinated erratically in both towel and soil tests at that time. They were held in dry cool storage and again germinated in July 1937. Since seeds were plentiful and accurate percentages of normal sprouts were desired, each sample was treated with Ceresan, 50-percent copper carbonate, copper stearate containing 17 percent by volume of New Ceresan, cuprous oxide, Merko, 17 percent of New Ceresan in talc, Smuttox, and zinc oxide. Although the exact data cannot be presented, it is sufficient to say that with the excess-dust method Ceresan, copper carbonate, Merko, New Ceresan, Smuttox, and zinc oxide provided a covering approximate to that obtained by 2 to 2½ ounces of dust per bushel of seed. Cuprous oxide did not adhere uniformly, ranging from 2 to more than 4 ounces per bushel and depending on the length of the agitation period and the thoroughness of sieving off the excess material.

Judging by the exhaustion of material from the treating flask, the adherence of the copper stearate-New Ceresan mixture was nearly double that of copper carbonate.

The relative chemical injury occasioned by these materials is shown in table 8. Undeniably the 83-percent copper stearate-17-percent New Ceresan mixture was extremely toxic to peas. As the weight column shows, the sprouts from seeds treated by this material were only three-fifths as heavy as the sprouts from Ceresan-treated seeds and about three-fourths as heavy as the sprouts from copper carbonate-treated peas. Both cuprous oxide and zinc oxide produced somewhat similar results in that the green weights of the sprouts were depressed in comparison with those of the Ceresan-treated tests. Smuttox by the same comparison appeared to have depressed the green weight of the sprouts. That the weight was not greater, however, was owing not to a toxic effect on the sprouts but to imperfect control of the fungus contaminants.

[5] Dr. G. W. Keitt contributed two dusts of this preparation.

TABLE 8.—*Relative chemical injury occasioned by various chemicals when used as laboratory dusts, and their effect on the germination and fungus contamination of pea seeds, and on the size and green weight of, and chemical injury to, the sprouts* [1]

Chemical applied as a dust	Germination	Fungus-contaminated seeds	Length of plumules	Length of radicles	Green weight of sprouts	Chemical injury	
						Sprouts	Tests
	Percent	*Percent*	*Inches*	*Inches*	*Grams*	*Percent*	*Number* [3]
Ceresan	92.8	0.3	2.5	2.9	47.3	1.3	4
Copper carbonate	87.9	2.0	1.9	1.5	39.2	51.1	36
Copper stearate with New Ceresan [2]	82.8	.3	.8	1.1	29.8	56.3	35
Cuprous oxide	89.9	2.2	1.7	1.2	36.3	65.2	37
Merko	93.1	3.8	1.9	2.8	44.2	1.8	6
New Ceresan	93.4	.2	2.2	2.8	45.4	.9	3
Smuttox	86.6	15.6	2.0	2.3	40.1	.7	3
Zinc oxide	90.7	3.1	1.7	1.4	36.4	58.8	36
Check	80.7	26.8	2.1	2.0	35.8	0	0

[1] All data are averages of 40 individual tests.
[2] A blended mixture of 1 volume of New Ceresan with 5 volumes of copper stearate.
[3] The number of tests in which chemically injured sprouts were observed.

When copper or zinc compounds are injurious the radicles of the seedlings, as explained by Crosier et al. (*5*), suffer relatively more than do the plumules. By using Ceresan rather than the fungus-rotted controls as a basis for comparison, the radicles of seeds in cuprous oxide-treated tests were reduced to two-fifths of the average normal length. Similarly, the plumules in the same tests were reduced to about two-thirds of the plumule length of the Ceresan-treated peas. The copper stearate-New Ceresan dust, however, reduced the lengths of the radicles to two-fifths, and that of the plumules to less than one-third of the respective portions of the Ceresan-treated sprouts. This latter type of seedling abnormality has been observed previously (*3, 13, 16*) in Ceresan- and New Ceresan-treated barley, oats, corn, and rye, and especially in tests of seeds treated with extremely large amounts of either chemical. Undoubtedly the injury in the studies with peas was also caused by excessive dosages of New Ceresan since three-fourths of an ounce of New Ceresan per bushel of seed is nearly the maximum noninjurious dosage for peas, and the actual rate in these experiments was 1 to 1½ ounces per bushel.

Another difference between copper and mercury injury to peas is the type of radicle abnormality. Toxic dosages of New Ceresan caused a shortening and often a thickening of the radicles. Usually the entire seedling appeared to have been decelerated in its growth. Copper-injured radicles were twisted, discolored, extremely brittle, and often without secondary roots. The root tips appeared to have been corroded or burned by a strong mineral acid. The copper injury was apparently permanent, the mercury injury in part was of a temporary nature.

The data in table 8 strikingly illustrate the value of the several mercury compounds in comparison with the other materials studied. New Ceresan when properly diluted with an inert carrier was conspicuously effective against common storage molds. Ceresan and Merko were less perfect fungicides, but the seedlings were normal and usually stronger than were those from the untreated peas. The abnormal seedlings in the copper- and zinc-treated rolls proved fully as troublesome in vitality determinations as did the fungus contami-

nations in the untreated rolls. Smuttox was not uniformly effective in mold control and never excelled Ceresan nor New Ceresan.

INSTANT DIP TREATMENTS

Although dust treatments appear to be justifiably preferred over dips or soaks for treatment of large bulks of seed, the opposite was true in seed laboratory work. The difficulty of obtaining a uniform dosage probably accounts for occasional injury and nonuniform control of molds in the dust applications. There was also considerable waste of material by the dry method. The diffusing dust particles were both disagreeable to, and dangerous when inhaled by the workers. An attempt was made to determine the correct concentrations of several materials in either suspension or solution in water so that they might be substituted for the dry materials.

TABLE 9.—*Influence of certain proprietary mercury dips on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the size and weight of, and chemical injury to, the sprouts*

BARBAK C

Variety or type of seed and concentration of dip	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests ratio [4]
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria				
Stored bulks:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
1.25 percent	92.5	2.8	0.8	3.4	4.9	0.7	1.6	41.1	12/15
Check	86.8	48.4	3.2	7.0	7.7	1.1	2.5	41.1	0/15
CERESAN									
Retail packets:									
1.0 percent	89.2	0.2	0	0	5.7			31.6	2/23
Check	76.9	43.3	1.9	18.1	15.8			27.4	0/23
1.0 percent, filtered [1]	80.8	.2	0	0	11.7	1.5	3.1	42.2	0/20
Check	77.8	24.7	5.9	0	15.8	1.4	2.9	39.9	0/20
World's Record:									
1.5 percent	84.8	1.4	0	0	4.4			30.8	2/15
Check	74.6	87.8	11.6	2.0	17.4			27.8	0/15
New-crop Idaho:									
1.5 percent, filtered [1]	84.1	[2]3	0	0	6.1	1.4	3.0	52.6	0/15
Check	84.3	8.1	18.2	17.0	8.0	1.3	2.9	50.7	0/15
World's Record:									
2.0 percent	85.4	1.0	.4	0	4.0			21.8	7/20
Check	76.4	53.0	13.8	0	14.8			16.0	0/20
New-crop Idaho:									
2.0 percent, filtered [1]	89.2	0	0	0	6.8	1.5	2.6	46.8	2/18
Check	86.5	4.3	1.0	2.5	8.2	1.5	2.7	47.9	0/18
MERKO									
Retail packets:									
1.5 percent	82.1	8.5	2.3	0	9.1				0/22
Check	80.7	13.3	4.0	0	13.4				0/22
New-crop canning:									
2.0 percent	89.5	.7	.2	0	6.6				0/25
Check	80.0	4.4	3.1	0	6.8				0/25
New-crop mixed:									
2.5 percent	92.3	2.8	2.5	1.7	1.0				0/20
Check	89.3	8.5	4.8	16.1	1.3				0/20
Garden varieties:									
3.0 percent	83.0	6.7	4.0	5.0	4.8	0.9	2.4	41.0	4/39
Check	82.5	13.7	5.5	24.2	5.0	.8	2.8	38.3	0/39

[1] Clear solution of ethyl mercury chlori le obtained by filtering off the nonsoluble carrier.
[2] Growth of *Ascochyta pisi* inhibited by treatment.
[4] See footnote 4, table 1.

TABLE 9.—*Influence of certain proprietary mercury dips on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the size and weight of, and chemical injury to, the sprouts*—Continued

NEW CERESAN

Variety or type of seed and concentration of dip	Germination	Seeds or sprouts contaminated with—				Length of plumules	Length of radicles	Green weight of sprouts	Chemically injured tests ratio
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria				
Commission boxes:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Grams*	
0.15 percent	80.1	1.2	0.1	0	10.7	.....	.....	39.8	0/32
Check	71.4	38.4	3.2	0	21.8	.....	.....	36.2	0/32
New-crop, 25 varieties:									
0.18 percent	93.1	.2	.1	0	2.2	.....	.....	27.7	1/65
Check	90.3	5.7	1.5	3.3	3.4	.....	.....	26.6	0/65
Stored seed:									
0.18 percent, decanted [3]	85.5	2.8	.7	0	7.2	.....	.....	21.8	0/25
Check	69.5	69.9	25.3	11.3	22.8	.....	.....	15.3	0/25
Retail packets:									
0.20 percent	80.2	2.0	.2	0	14.6	1.0	2.0	49.0	2/19
Check	76.6	34.1	7.5	0	18.5	1.0	1.9	49.3	0/19
Year-old bulks:									
0.20 percent, filtered [3]	88.0	.4	.1	0	5.3	.7	1.8	47.4	0/20
Check	81.7	31.2	4.5	2.5	15.1	.7	1.8	47.0	0/20
Commission boxes:									
0.25 percent	66.0	3.7	1.3	0	28.2	.7	1.5	44.5	4/33
Check	60.8	61.2	23.0	20.4	37.7	.8	1.4	43.3	0/33
New-crop garden:									
0.25 percent filtered	84.2	0.3	.5	0	10.6	.7	1.8	47.2	1/10
Check	81.7	2.6	7.0	2.7	12.1	.7	1.4	46.6	0/10
Commission boxes:									
0.30 percent	54.2	2.4	1.3	0	42.7	.9	1.9	49.6	2/11
Check	44.9	94.3	19.1	0	52.3	.8	1.8	48.9	0/11
Year-old bulks:									
0.30 percent filtered	91.2	1.3	.2	0	2.0	1.1	2.2	56.9	2/12
Check	84.7	38.3	4.4	10.9	9.1	1.0	2.3	55.3	0/12

SANOSEED

Retail packets:									
1.5 percent	69.9	30.4	2.1	0	13.8	.....	.....	36.6	0/30
Check	56.2	86.5	5.4	0	28.5	.....	.....	32.9	0/30
Storage bulks:									
2.0 percent	81.5	4.7	.4	0	8.6	.....	.....	34.0	2/16
Check	76.6	55.0	1.5	0	15.0	.....	.....	34.0	0/16

[3] Clear solution of ethyl mercury phosphate obtained by decanting or by filtering off the nonsoluble talc carrier.

PROPRIETARY MERCURIALS

Suspensions of New Ceresan were tested extensively at concentrations of 0.15 to 0.30 percent. The results, summarized in table 9, indicate that these concentrations are effective disinfestants and are not toxic to pea sprouts. The 0.18-percent concentration was selected by the State seed-testing laboratory for use in treating peas, beans, and corn. The amount of ethyl mercury phosphate adhering to the seeds was nearly identical with that obtained by the dry method when employing a 17-percent New Ceresan dust.

Hofer and Hamilton (*6*) used an aqueous suspension of only 0.125 percent of New Ceresan for disinfesting legume seeds and reported satisfactory control of fungi. Recent work in the seed-testing laboratory with vetch-seed disinfestation was successful with a 0.30-percent instant dip. Very little injury was noted when the seeds were transferred directly to agar plates, and the percentage of sterile transfers significantly exceeded those from a chlorine solution.

The dip and dust equivalents for New Ceresan having been determined, the correct concentrations for other materials were readily computed. The substitution of dip for dust treatments with other mercury compounds has not, however, always been successful. The mercurial in Ceresan is soluble and has given uniformly good disinfestation, but Merko and Sanoseed (table 9) have not. It is difficult to keep these materials in suspension, and the mercury adhering to each seed cannot be accurately controlled. In laboratory practice it is necessary to pour the material over the seeds several times or to agitate the beaker containing the seeds and the suspension. Based on the effective dosage of New Ceresan, a 1-percent suspension of Ceresan, Merko, or Sanoseed should prevent development of saprophytes, but it did not always do so.

For a time a 0.9-percent Ceresan suspension was regularly used in routine testing of certain vegetable and flower seeds. Profuse growths of *Rhizopus nigricans* were eliminated, although *Penicillium* spp. and bacteria persisted on the dead seeds. Increases in the strength of Ceresan up to 1.5 or even 2.0 percent effected only a slightly better average control but did prevent the occasional development of saprophytes on highly contaminated seed stocks. Apparently this is true for peas as well. Since the application of a 1.5-percent suspension was not accompanied by any definite injury, it is to be preferred over a weaker one.

Barbak C was not studied extensively, but readings from treated and untreated samples of 15 varieties of peas showed satisfactory control of fungi. *Rhizopus nigricans* was easily eliminated, but surprisingly enough *Dematium pullulans* appeared to tolerate a 1.25-percent suspension of this material. The unfiltered 1.25-percent suspension proved toxic to a high percentage of the sprouts. The injured seedlings were very similar to those developing from peas treated with an excess of New Ceresan. That the green weight of the treated tests should equal that of the untreated tests although the phytocidal action was very marked, may appear paradoxical. It should be remembered, however, that the number of sprouts was greater in the Barbak C-treated than in the untreated tests. Furthermore, while growth in the length of both plumules and radicles was retarded by the suspension, the radial growth of the sprouts developing from the treated seeds exceeded that of the untreated sprouts.

Filtered and Decanted Solutions of Mercurials

In preliminary dip treatments care was taken to agitate the suspensions so that the seeds would obtain a uniform coverage. In later work filtrates or the decanted supernatant liquids were substituted. There was little difference in control of micro-organisms and development of sprouts between the suspension types and filtrate types of Ceresan or New Ceresan. The data in table 9 express the similarity very clearly. Apparently the active medicants, ethyl mercury chloride and ethyl mercury phosphate, were readily soluble in water. Removal of the filtrates from Merko and Sanoseed suspensions depleted their fungicidal value. In several trials the percentages of contaminated seeds were greater in the treated than in the control tests probably because of dissemination of spores in the liquids.

Considerable difficulty was encountered in maintaining a uniform suspension of Barbak C. The inert diluent tended to accumulate on

the surface of water and repeated agitation of the sample and water was necessary to obtain a uniform covering. In later experiments the diluent was removed by filtering. The filtrates from 1.0- and 1.25-percent suspensions had no effect on the fungus or bacterial contaminants. Apparently the active ingredient, phenyl mercury cyanamide, was insoluble in water and was filtered off with the diluent.

Inorganic Mercury Compounds

Mercuric chloride has been used for years as a fungicide. Usually the tissue to be surface-sterilized is immersed in a mercuric chloride solution for a definite limited period and is then thoroughly rinsed in water to remove the chemical. This procedure is not practical in routine seed testing. The time element alone is an obvious disadvantage. The period of soaking, unless exceedingly brief, would permit the impoverishment of the mercury content of the solution and necessitate frequent replacements. Removal of the disinfestant after sterilization, moreover, is neither necessary nor desirable since an extended protective action is essential in a crowded spore-laden germinator.

For these reasons an instant-dip method of treating appeared advantageous. Since it had been established that a dilution of mercuric chloride dust affected a control of contaminants almost identical with that obtained by equal dilution of New Ceresan, it appeared likely that similar aqueous concentration would produce comparable control. Thus an 0.18-percent solution of mercuric chloride should be interchangeable with the equivalent suspension of New Ceresan which proved effective in the disinfestation of, and protection to, pea seeds. Laboratory practice has verified this.

A few of the pertinent data of many separate tests are assembled in table 10. Apparently a concentration as low as 0.06 percent is at times an effective fungicide. Sprouts were not appreciably injured until an 0.25-percent concentration was reached. It should be emphasized that all of the results were obtained from instant dips at 70° F., that the seeds were never washed, and that they were placed to germinate immediately. Very probably lower concentrations used as brief soaks would be effective.

In dipping seeds in mercuric chloride solution a small volume, usually 100 or 150 cc., was poured into a glass tumbler and used repeatedly until exhausted. Even when 40 successive lots of 100 seeds each were dipped the concentration of the active chemical apparently was not seriously depleted. Control of fungi was as complete for the last as for the first lot of peas dipped in a 0.15- or 0.18-percent solution. An original concentration of only 0.06 or 0.10 percent might not have given similar results since a slight impoverishment of the solution would have reduced the mercury content to a nonpathogenic strength. Because mercuric chloride is nonvolatile and is also soluble in water it is ideal for laboratory control of saprophytes. In order to compare results with another inorganic mercury, mercurous chloride was used in a few trials. The obvious disadvantage of low solubility in water curtailed extended work with this chemical. Suspensions of 1.0 and 0.5 percent were tested with new stocks of canning peas, giving results as averaged in table 10. The more concentrated suspension compared favorably with other mercury compounds, but the weaker possessed little fungicidal value. No injury was noted in either suspension. A

slightly increased concentration could presumably be employed safely and possibly with complete elimination of fungus contaminants.

TABLE 10.—*Influence of solutions of mercuric chloride and suspensions of mercurous chloride on the germination and on the saprophytic and bacterial contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

MERCURIC CHLORIDE

Variety or type of pea seed and concentration of chemical	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests (ratio) [1]
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria		
Surprise:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
0.06 percent	98.0	0	0.2	0	0.2		0/20
Check	96.8	3.2	.6	1.0	.8		0/20
Canning varieties:							
0.10 percent	88.1	4.0	5.1	.1	6.1	48.6	0/15
Check	83.2	50.4	21.8	12.7	13.5	50.5	0/15
Stored bulks:							
0.18 percent	87.2	.4	.2	0	7.9	45.7	0/30
0.18 percent New Ceresan	89.0	.1	0	0	6.2	47.2	0/30
Check	81.9	29.2	.9	11.3	11.6	42.4	0/30
New-crop, western:							
0.20 percent	95.0	.5	.2	.1	1.8	45.6	4/35
Check	95.2	13.1	.8	29.8	3.8	45.7	0/35
0.25 percent	93.4	.2	.1	0	.7	38.7	11/24
Check	94.3	6.8	.7	62.6	1.8	45.1	0/24
0.30 percent	93.5	.1	0	0	1.3		8/15
Check	93.0	12.1	2.7	49.3	2.3		0/15
Stratagem:							
0.40 percent	91.7	.5	0	0	2.8		3/11
Check	90.1	12.2	4.8	1.8	6.7		0/11

MERCUROUS CHLORIDE

Canning varieties:							
0.5 percent	89.9	27.2	1.7	55.5	4.8	50.4	0/10
Check	92.6	29.5	.9	64.0	3.7	49.1	0/10
1.0 percent	94.1	2.2	.8	15.9	1.5	49.2	0/20
Check	93.3	15.9	1.4	27.8	8.5	47.4	0/20

[1] See footnote 4, table 1.

COPPER COMPOUNDS

The majority of the copper compounds recommended for seed protection are not highly soluble in water. Copper sulphate is the most notable exception and should perhaps be regarded as a disinfectant rather than as a protectant. It was first employed in preference to other copper compounds largely because of the ease of dipping seeds in a solution. As may be observed from the data in table 11, the fungicidal-phytocidal ratio of this chemical is extremely low. New Ceresan or mercuric chloride effected significantly better control of fungi and bacteria and without injury to the sprouts. Copper sulphate did not depress the apparent germination of peas but did prevent the normal development of the seedlings. The green weight of the sprouts from the treated seeds rarely equaled that of sprouts in the untreated rolls.

Suspensions of copper oxychloride only slightly reduced the percentages of fungus-contaminated sprouts. The results presented in table 11 indicate that extremely concentrated suspensions would be required to effect significant fungicidal action. Repeated agitation was necessary to maintain a uniform concentration.

TABLE 11.—*Influence of various dip treatments with copper compounds and certain other chemicals on the germination and on the saprophytic contaminants of pea seed, and on the green weight of, and chemical injury to, the sprouts*

Variety or type of pea seed and concentration of chemical	Germination	Seeds or sprouts contaminated with—				Green weight of sprouts	Chemically injured tests, ratio [1]
		Rhizopus nigricans	*Penicillium* spp.	*Dematium pullulans*	Bacteria		
Stored 1 year:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	
1.0-percent copper hydroxide	89.3	29.8	1.0	0	6.4	39.1	0/16
0.18-percent New Ceresan	91.4	1.6	0	0	5.4	40.2	0/16
Check	81.4	71.5	3.3	0	15.6	36.2	0/16
New-crop, garden:							
2.0-percent copper hydroxide	90.7	11.6	1.0	12.0	3.3	39.4	8/19
Check	89.4	24.8	1.2	39.0	4.2	43.3	0/19
New-crop, mixed:							
0.5-percent copper oxalate	93.0	6.0	1.5	0	4.0	.....	0/15
1.0-percent copper oxalate	91.5	5.0	2.4	0	4.8	.....	0/15
Check	94.5	8.0	4.0	0	3.2	.....	0/15
New-crop, garden:							
1.0-percent copper oxychloride	83.9	14.6	3.7	2.0	11.3	.....	0/28
0.2-percent New Ceresan	84.8	.3	.4	.5	5.2	.....	0/28
Check	82.8	22.6	9.7	10.6	10.8	.....	0/28
New-crop, canning:							
1.5-percent copper oxychloride	92.4	4.7	1.2	3.6	.8	.....	1/15
0.18-percent New Ceresan	91.7	0	.1	0	.7	.....	0/15
Check	91.1	5.7	.6	44.4	1.1	.....	0/15
Retail packets:							
3.0-percent copper oxychloride	87.5	13.0	7.0	0	8.0	.....	1/10
3.0-percent Sanoseed	88.0	6.5	3.9	0	2.5	.....	0/10
Check	86.5	22.0	11.5	0	8.0	.....	0/10
Canning stocks:							
0.33-percent copper sulphate	96.0	2.0	0	0	1.0	.....	10/20
0.18-percent New Ceresan	96.5	0	0	0	0	.....	0/20
Check	94.4	5.0	1.0	0	1.6	.....	0/20
New-crop, mixed:							
1.0-percent copper sulphate	92.9	11.1	.5	.4	1.8	.....	21/22
0.15-percent New Ceresan	94.0	1.1	0	0	1.4	.....	0/22
Check	92.7	10.9	2.7	2.4	3.9	.....	0/22
Garden varieties:							
2.0-percent cuprous oxide	83.8	6.2	2.0	2.5	5.2	28.5	15/30
Check	90.0	39.5	1.5	41.2	5.0	40.0	30/30
Stored bulks:							
5.0-percent ferrous sulphate	45.0	91.7	30.0	0	42.8	30.5	1/8
Check	69.9	78.6	10.6	0	28.0	45.0	0/8
Stored packets:							
10.0-percent ferrous sulphate	39.8	85.3	45.4	0	57.6	28.8	16/16
Check	76.9	53.9	11.2	0	20.5	40.7	0/16
Canning bulks:							
2.0-percent potassium dichromate	72.8	58.4	6.3	0	23.4	42.9	3/10
Check	73.1	73.5	4.0	16.3	21.6	39.7	0/10

[1] See footnote 4, table 1.

Copper oxalate, copper hydroxide, and cuprous oxide were also studied as dips for several varieties of peas. They remained in suspension for only a few minutes unless disturbed. Usually the seeds were dropped into a beaker containing the aqueous dip and the entire contents were then poured from one beaker to another to obtain a uniform coverage. The results for the individual rolls of peas indicated, however, that the dosages were not identical. Cuprous oxide severely injured the seedlings in a number of the tests while in others the sprouts developed normally. The other copper compounds appeared less injurious but were also less effective in reduction of contaminations.

OTHER CHEMICALS

From time to time a number of chemicals not employed generally as fungicides were tried as laboratory disinfestants or protectants. With practically no exceptions solutions or suspensions of metallic salts either injured the seedlings or failed to control the saprophytes. Potassium iodide seemed promising at first, but the cost of the ma-

terial curtailed extensive experimentation. Borax and sodium hypochlorite, which have been suggested as fruit washes, proved of little value. The former material did not stay in solution in cold water, while the latter was not effective against molds when used as an instant dip.

Solutions of iron compounds were extremely toxic to the seedlings and actually increased the percentages of contaminated seeds. As shown in table 11 there is no possibility of utilizing ferrous sulphate for protection of contaminated germinants. Potassium dichromate, however, actually increased the green weights of developing sprouts. Solutions of both ferrous sulphate and potassium dichromate discolored the working surfaces as well as the paper rolls.

DISCUSSION

The chemical treating of peas has facilitated the reading of germination tests. The time consumed in applying the chemical has been compensated for by the rapid examinations of the uncontaminated seedlings. The repeating of tests injured by saprophytes has been entirely obviated, resulting in a saving of germinator space and a decrease in the rush periods of seed routine testing. Excessive applications of water, particularly at temperatures above 20° C., encourage fungus and bacterial growth. Untreated samples have often been ruined under these conditions. It has been found that the variations in watering and temperature have little effect on treated peas. Furthermore, when necessary the usual 6-day germination period can safely be prolonged without danger of profuse development of *Rhizopus nigricans*.

In laboratory practice samples of peas were always germinated in duplicate, one treated, and the other untreated. Comparisons of contaminations, strong sprouts, and seedling development were made from the two rolls placed side by side on a tray and therefore were very exact. In severely contaminated samples it was evident which rolls contained untreated seeds. At the end of the germination period *Rhizopus nigricans* entirely obscured the untreated tests while the alternating rolls of treated seeds appeared clean. Mycelium occasionally grew from the untreated into the treated tests. Since only the chemical applied to the seeds was available to protect the seedlings a very severe test of the chemical was created. Under no conditions were peas treated with 0.18-percent New Ceresan, 0.20-percent mercuric chloride, or 1.5-percent Ceresan dip contaminated to any extent. Other mercury compounds were nearly as efficient. The copper and zinc compounds protected the seeds, but the sprouts which developed were often covered by mycelium. Apparently the low rate of solubility of the copper and zinc compounds limited the active zone to the points of application, whereas, the soluble materials, New Ceresan, mercuric chloride, and Ceresan, protected the seedlings as well.

The materials included in this work did not eliminate several fungi. *Alternaria* spp. apparently increased both in prevalence and severity after seed treatment. It seemed certain that in part the visible increase was caused by lack of obscuring growths of *Rhizopus nigricans*. This was very noticeable in several samples dipped in New Ceresan solutions. The percentage of *Alternaria*-infected seeds of certain kinds of flowers appeared to have been doubled after applications of

mercuric chloride, cuprous oxide, and New Ceresan. *Rhizoctonia solani* developing from a single infected seed spread throughout and killed entire rolls of peas treated with Ceresan. The growth of *Fusarium* spp. was slightly inhibited, and the infection of adjacent sprouts was prevented by many materials. Jones (*9*) found that *Ascochyta pisi* is tolerant of organic mercurials. In the laboratory, the number of seeds with visible mycelium was reduced from six to one in a sample of Alderman peas and apparently increased in samples of Telephone and of Thomas Laxton which had been treated by New Ceresan. Lack of control was observed also in mercuric chloride-, mercurous chloride-, and Barbak C-treated seeds. Evidently the action of these materials was limited to protection, disinfestation, and very slight disinfection.

Certain seed growers have expressed a preference for sand, soil, or other solid substratum. They contend that contact contamination by micro-organisms is minimized resulting in accurate percentages of germinations. To determine whether evaluations of normal sprouts in towels were approximately similar to soil tests a number of samples were tested in towels and in soil. Neither sand, pasteurized soil, nor muck increased the percentages of the apparent strong sprouts over treated seed in towels. Various chemicals were more instrumental in accentuating percentages of germination.

In any sample of peas containing dead seeds are also seeds which are viable but which easily succumb to the organisms commonly termed "saprophytes." When protected by a chemical or when planted in sterile soil, normal seedlings may develop. If, however, the seeds are contaminated, the chemical functions as a disinfectant and disinfestant as well as a protectant. The original fungus associates are of no moment except insofar as they injure the dry seed. Without the chemical applications, the seeds, whether isolated in soil or on towels, usually do not produce normal sprouts. For this type of seed-stock, testing of treated seeds in towels yields more exact germination data.

While this study was undertaken to determine the value of certain materials as seed disinfestants in laboratory seed-germination practice, it might well serve to anticipate their probable success in field experiments. Questions of stimulations, toxicity, and specific protection from certain organisms could be given a visual approach. Materials which are not injurious to seeds in towel tests could be safely applied to peas planted in any type of soil. That chemicals which caused stunting and weight reduction in towels do not do so in most soils suggests that a portion of the dosage is sterilizing the surrounding soil, or at least, is removed from contact with the seedlings. Much as the glass slide (*15*) was found to be useful in comparing fungicides designed for foliage protection, so can germination trays and towels serve to guide pathologists in the use of seed treatments for the field.

SUMMARY

Germination of pea seeds (*Pisum sativum* L.) in moistened paper towels permits certain seed-borne saprophytes to develop profusely. The pea seedlings may be so severely injured that the seed stocks cannot be appraised accurately.

Since crowded germinator facilities prohibit wide spacing of seeds as a means of isolating saprophytes, various chemicals were studied for inhibiting the growth of seed contaminants.

Seed peas varying from those practically free of any micro-organisms, to others carrying a high contaminant load were treated with both wet and dry applications of different materials. The value of a chemical was based on its inhibiting action to contaminants, phytotoxicity, ease and rapidity of application, health hazards, and cost.

Ethyl mercury phosphate effectively protected both seeds and seedlings from the seed-borne fungi *Rhizopus nigricans*, *Penicillium* spp., *Dematium pullulans*, and to a slight extent from soft-rotting bacteria. New Ceresan inhibited saprophytes even when diluted with 10 times its bulk of an inert material.

Storage of New Ceresan-treated seeds to allow volatilization of ethyl mercury phosphate was not necessary for fungus control.

Other proprietary dusts containing volatile mercurials inhibited growth of contaminants.

The radicles of Ceresan-treated peas were consistently longer than those of untreated seeds. The green weights of the sprouts was also greater for the treated peas.

For laboratory work nonvolatile mercury dusts were preferred to materials containing volatile mercuries, because of the lesser health hazard of the former.

The proper dilution of mercuric chloride with an inert dust compared favorably with New Ceresan in respect to control of contaminants. Mercurous chloride was difficult to apply uniformly to the peas and frequently injured the seedlings. Reduced phenyl mercury nitrate disinfested most of the lots of peas.

Undiluted cuprous oxide severely injured radicles while dilutions which were not phytotoxic failed to control fungi.

Copper stearate rarely induced any abnormality in seedling development. Mixtures of copper stearate with copper carbonate, copper sulphate, or New Ceresan were extremely injurious to pea sprouts.

A dust containing 80 percent of copper stearate, 16 percent of diluent, and 4 percent of New Ceresan completely disinfested 30 varieties of peas.

Zinc chromate and zinc stearate were less injurious than zinc oxide.

Sulphur and volatile aldehydes were not dependable in reducing fungus-seed associations.

Injury to seedlings occasioned by copper compounds was limited to deformation, discoloration, or killing of the radicles. Excessive dosages of mercurials usually caused a deceleration in elongation of both plumules and radicles.

Dip treatments with aqueous suspensions or solutions were safer, more easily controlled, faster to apply, and less wasteful of materials than were dust treatments.

Both mercuric chloride and New Ceresan at concentrations of 0.18 percent consistently eliminated contaminants and usually were effective at lower concentrations.

Dilutions of 0.9 to 1.5 percent of Ceresan controlled fungi with no depressing effect on germination.

Mercurous chloride and the slightly soluble copper compounds did not effect uniform fungus elimination since they were difficult to maintain in suspension.

Certain chemicals used as fruit washes were too evanescent to protect pea seedlings during the 6-day germination period.

Alternaria spp., *Fusarium* spp., and *Rhizoctonia solani* were tolerant of all of the chemicals studied. Elimination of *Rhizopus nigricans* permitted *Alternaria* spp. to develop rapidly and also made its recognition easier.

Ethyl mercury phosphate occasionally decreased the percentage of *Ascochyta pisi*-infected seeds.

The testing of chemically treated peas in towels yielded more exact germination data than untreated seeds widely spaced in pasteurized soil.

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EFFECT OF BORDEAUX MIXTURE AND ITS VARIOUS ELEMENTS ON TRANSPIRATION[1]

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INTRODUCTION

That bordeaux mixture may increase transpiration has long been established, but the recent series of dry summers in the United States has focused the attention of investigators on this effect of bordeaux mixture. Some of the early workers considered that bordeaux mixture decreased transpiration; later it was concluded that it accelerated transpiration. As late as 1931, however, Miller (*11*)[3] concluded that bordeaux gave little or no increase under the conditions of his tests. Obviously bordeaux transpiration[4] is not completely understood.

OBJECT OF THE INVESTIGATION

A study of bordeaux transpiration was made in the greenhouse between December 1935 and June 1936 in connection with an extensive series of experiments on the fungicidal possibilities of several copper compounds, especially red cuprous oxide. Inspection of the data obtained revealed that they were in general agreement with the finding that bordeaux mixture increases the rate of water loss. The data varied from published reports at several points, however.

Speculation upon the correlations and discrepancies in the available data led further and further into the causes of bordeaux transpiration until finally a theory was evolved to account for essentially all of the known facts. The writers' data are sufficient to rough in this theory of bordeaux transpiration, but they are augmented by the published data of other investigators, particularly Wilson and Runnels whose classical set of data on bordeaux transpiration in the publications here cited have facilitated the formulation of this theory.

Several other aspects of bordeaux injury have also been investigated recently at this station. It has been interesting to observe that the various elements in bordeaux bear essentially the same relation to each other whether one studies transpiration, leaf deformation, dwarfing, or defloration[5] (*6*).

MATERIALS AND METHODS

Tomatoes (*Lycopersicum esculentum* Mill.) proved to be good indicator plants for experiments on the recovery of seedlings that

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[3] Italic numbers in parentheses refer to Literature Cited, p. 442.
[4] The term "bordeaux transpiration" has been telescoped from the expression "the effect of bordeaux mixture on transpiration of plants." This condensation gains much in simplification, but loses nothing in comprehension.
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were transplanted immediately after spraying. The variety was a pure-line stock of John Baer. The irregular leaves, however, were unsatisfactory for leaf measurements in experiments where water loss was to be calculated on the basis of leaf area. For such experiments beans (*Phaseolus vulgaris* L.) were used because bean plants had already been used extensively in other transpiration work (*4*). The bean seed was a pure-line stock of Stringless Green Pod Refugee.

Experimental plants were grown in a greenhouse where the air temperature was maintained at 24° C. by a thermostatically regulated steam valve and blower. The temperature control was satisfactory during the night and even on partly cloudy days until April, but the blower in the system proved incapable of handling the heat input on days of brilliant sunshine. In the latter case the temperature rose 5° to 10° above the setting.

For the major part of the work which was conducted with beans, the seeds were sown in pasteurized greenhouse loam in double-walled irrigators previously described (*5*). Irrigators rather than ordinary crocks were used to assure more uniform distribution of water to the roots of the plants. Between tests the soil was removed, the irrigators were treated with 1–100 formalin, and new pasteurized soil was added.

In the first few tests, the bean seedlings were thinned to one in each irrigator, but in later experiments three were left in each pot to reduce plant variability and to give larger leaf area.

As soon as the simple leaves were fully expanded, the surface of the soil was covered with a mixture of paraffin and beeswax (4 parts paraffin and 1 part beeswax) to prevent loss of water by evaporation from the surface of the soil. Small-mouthed bottles were used as reservoirs, one to each irrigator. The soil-moisture system operated with a capillary tension of 17.5 cm of water. The soil thus remained quite moist.

The transpiration losses for the preceding 24 hours were determined by replacing the water taken from the irrigators at 8 a. m., but no readings were taken until the moisture content of the soil had reached equilibrium after the pots were sealed. Readings were taken 2 to 4 days before sprays were applied so that the plants could be arranged into groups of approximately equal transpiration rates.

Most published data on bordeaux transpiration deal with simple differences in water loss between sprayed and nonsprayed plants. In these experiments the unit of leaf area has been taken as a basis for calculating water losses although there are certain difficulties in the calculation.

The difficulties arise because a leaf has two surfaces of equal area which lose water at different rates, and which are affected differently by sprays. When water loss is measured by replacement, the figure represents the sum of the loss from the upper and lower surfaces. For that reason it is common to use the planimeter reading in calculation. Doubling the planimeter reading would give more weight to the effect of the two surfaces, but this would not change the result mathematically and would not be any more accurate because the two surfaces lose water differently.

The data in this paper are expressed on the basis of the area of one side, but this basis must be kept in mind in studying data from tests where the two leaf surfaces were sprayed separately. The area of bean leaves can be calculated from the product of the width and the

length by a simple formula already worked out (*4*). Leaves were measured every 4 days. Each measurement was taken as the basis for calculating the amount of water transpired per unit of surface area for the 2 days preceding and the 2 days succeeding the measurement.

Since the time when Duggar and Cooley's work was published (*3*), most data have been calculated as a ratio of sprayed to nonsprayed, the "transpiration ratio" as Martin and Clark (*10*) call it. This method of calculation seems sound when referring two treatments to the same check. It may lead to erroneous conclusions, however. Since the transpiration of nonsprayed plants is the denominator of the fraction used in calculating transpiration ratios, any shift in the size of the denominator will shift the ratio without any alteration in the numerator, which, of course, is the transpiration of the sprayed plants. Therefore, as long as the size of the denominator remains constant as in comparing treatments within one experiment, conclusions based on transpiration ratios are sound. Since the size of the denominator shifts with the weather, transpiration ratios for different weather conditions or for different experiments must be used with caution.

The data on transpiration per square centimeter of leaf area (one side) are given in the tables as transpiration differences (sprayed minus nonsprayed) and as transpiration ratios (sprayed divided by nonsprayed). A correction is applied for any prespray differences in water loss for the various plants.

The basic sprays used were bordeaux 4–4–50 (4 pounds $CuSO_4$, 4 pounds $Ca(OH)_2$, 50 gallons water) and electrolytic red cuprous oxide 1–50, unless otherwise stated. These sprays contain approximately 0.25 percent of copper. Cottonseed oil, lime, and Bancroft clay were sometimes used for comparative purposes. Unless otherwise stated the lime was newly burned in a muffle furnace to assure freedom from carbonation, a factor found to be of considerable importance in this problem. The cottonseed oil was emulsified by shaking it in a flask with 0.5 percent of sodium lauryl sulphate.

Each group of plants was sprayed in the morning, a deVilbiss atomizer being used unless otherwise stated. The irrigators were then distributed over a center greenhouse bench so as to minimize differences in position. No clinostat was available.

RESULTS AND DISCUSSION

In arriving at a general theory of bordeaux transpiration, the various components of the spray mixture have been isolated and studied separately as far as possible. Data are presented also on influence of weather, time, and chemical and physical qualities of bordeaux. The differential effect of bordeaux on the cuticle and the stomata is also discussed. Data on each point are discussed in turn leading up to a final discussion of an hypothesis for bordeaux transpiration.

EFFECT OF WEATHER FACTORS

Under the conditions of these experiments sprays caused greater transpirational increases on beans when they were applied on bright days when the leaves were in direct sunlight than when they were applied on dull days (table 1).

TABLE 1.—*Effect of various sprays on transpiration of beans*

[Water loss expressed as milligrams per square centimeter per day [1]]

EXPERIMENT NO. 4. SPRAYS APPLIED BRIGHT DAY FEB. 6, 1936: BORDEAUX 4-4-50; RED CUPROUS OXIDE 1-50; RED CUPROUS OXIDE 1-50+COTTONSEED OIL ½ PERCENT; LIME 4-50; CLAY 4-50; COTTONSEED OIL ½ PERCENT

Type and date of record	Weather	Water loss for nonsprayed plants	Bordeaux		Red cuprous oxide						Lime						Cottonseed oil		Bancroft clay	
					Alone		Plus clay		Plus oil		Pure		Carbonated		Pure + clay					
			Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]
Standardizing period	Fair	110.5	112.9	.....	113.3	.....	.....	.....	119.1	.....	.....	.....	117.8	.....	.....	.....	112.1	.....	.....	.....
Correction applied	.....	.....	−2.4	.....	−2.8	.....	.....	.....	−8.6	.....	.....	.....	−7.3	.....	.....	.....	−1.6	.....	.....	.....
Feb. 6	Fair	104.2	194.1	87.5	106.4	−0.6	.....	.....	115.8	3.0	.....	.....	117.2	5.7	.....	.....	115.4	9.6	.....	.....
Feb. 7	Snow	106.6	148.6	39.6	113.6	4.2	.....	.....	108.1	−7.1	.....	.....	118.7	4.8	.....	.....	105.2	−3.0	.....	.....
Feb. 8	....do....	103.0	149.2	43.8	112.2	6.4	.....	.....	103.7	−7.9	.....	.....	113.0	2.7	.....	.....	98.5	−6.1	.....	.....
Feb. 9	....do....	90.3	123.2	30.5	105.8	12.7	.....	.....	98.1	−.8	.....	.....	104.4	6.8	.....	.....	90.0	−1.9	.....	.....
Feb. 10	....do....	95.9	118.3	20.0	109.6	10.9	.....	.....	92.3	−12.2	.....	.....	107.2	4.0	.....	.....	91.3	−6.2	.....	.....
Feb. 11	Fair	125.0	147.7	20.3	139.0	11.2	.....	.....	123.9	−9.7	.....	.....	138.2	5.9	.....	.....	118.2	−8.4	.....	.....
Average	.....	104.2	146.8	40.3	114.4	7.4	.....	.....	107.0	−5.8	.....	.....	116.4	4.9	.....	.....	103.1	−2.7	.....	.....
Corrected transpiration ratio	.....	.....	.....	138.6	.....	107.1	.....	.....	.....	94.4	.....	.....	.....	104.7	.....	.....	.....	97.4	.....	.....	.....

EXPERIMENT NO. 6. SPRAYS APPLIED DULL DAY MAR. 9, 1936: BORDEAUX 4-4-50; RED CUPROUS OXIDE+CLAY 1-7-50; LIME 8-50; LIME+CLAY 4-4-50; CLAY 8-50

Standardizing period	Fair	149.0	139.5				148.0				150.0		147.5		137.5			156.0	
Correction applied			9.5				1.0				−1.0		1.5		11.5			−7.0	
Mar. 9	Cloudy	124.0	143.0	28.5			127.0	4.0			133.0	8.0	123.0	0.5	125.0	12.5		118.0	−13.0
Mar. 10	do	153.0	151.0	7.5			158.0	6.0			163.0	9.0	154.0	2.5	149.0	7.5		166.0	6.0
Mar. 11	Rain	69.0	72.0	12.5			73.0	5.0			70.0	.0	68.0	+.5	70.0	12.5		72.0	−4.0
Mar. 12	Snow	51.0	53.0	11.5			63.0	13.0			61.0	9.0	57.0	7.5	57.0	17.5		64.0	6.0
Mar. 13 [3]	do	147.0	164.0	26.5			137.0	−9.0			149.0	1.0	122.0	−23.5	140.0	4.5		155.0	1.0
Mar. 14	do	167.0	156.0	−1.5			168.0	2.0			175.0	7.0	151.0	−14.5	162.0	6.5		174.0	.0
Mar. 15	Fair	183.0	166.0	−7.5			177.0	−5.0			191.0	7.0	171.0	−10.5	175.0	3.5		194.0	4.0
Average		127.7	129.3	11.1			129.0	2.3			134.6	5.9	120.9	−5.3	125.4	9.2		134.7	.0
Corrected transpiration ratio			108.6				101.1				104.6		95.8		107.2			100.0	

EXPERIMENT NO. 7. SPRAYS APPLIED BRIGHT DAY APR. 4, 1936: BORDEAUX 4-4-50; RED CUPROUS OXIDE 1-50; RED CUPROUS OXIDE+CLAY 1-7-50; LIME 8-50; LIME+CLAY 4-4-50; CLAY 8-50

Standardizing period	Snow	167.3	161.6		170.0		161.0				173.1		171.7		156.7			167.2	
Correction applied			5.7		−2.7		6.3				−5.8		−3.4		10.6			.1	
Apr. 4	Fair	226.9	291.1	69.9	232.0	2.4	240.3	19.7			265.1	32.4	235.8	5.5	244.7	28.4		263.3	36.5
Apr. 5	Cloudy	231.7	254.3	28.3	242.9	8.5	239.1	13.7			267.8	30.3	233.2	−1.9	242.9	21.8		263.2	31.6
Apr. 6	Rain	98.8	116.7	23.6	99.9	−1.6	114.9	22.4			115.1	10.5	103.7	1.5	100.3	12.1		119.4	20.7
Apr. 7	Cloudy	113.8	137.6	29.5	118.6	2.1	128.7	21.2			137.2	17.6	124.2	7.0	121.1	17.9		136.1	22.4
Apr. 8	Fair	192.0	228.6	42.3	194.7	.0	201.7	16.0			230.5	32.7	203.1	7.7	199.7	18.3		214.8	22.9
Apr. 9	Cloudy	149.0	180.0	36.7	147.7	−4.0	174.8	32.1			181.4	26.6	165.0	12.6	160.3	21.9		176.1	27.2
Average		168.6	201.4	38.4	172.6	1.2	183.3	20.9			199.5	25.1	177.5	5.4	178.2	20.1		195.5	26.9
Corrected transpiration ratio			122.8		100.8		112.4				114.9		103.3		112.0			116.0	

[1] Water loss read at 8 a. m. Readings given were taken the day after.
[2] Transpiration difference is sprayed minus nonsprayed corrected for differences observed during standardizing period.
[3] Effects of second spray show here.

TABLE 2.—*Effect of spraying upper and lower leaf surface on transpiration of bean leaves*

[Water loss expressed as milligrams per square centimeter per day [1]]

EXPERIMENT NO. 8. SPRAYS APPLIED ON BRIGHT DAY MAY 1, 1936: BORDEAUX 4-4-50; RED CUPROUS OXIDE 1-50; OIL ½ PERCENT

Type and date of record	Weather	Water loss for nonsprayed plants	Bordeaux						Red cuprous oxide						Cottonseed oil			
			Both surfaces		Upper surface		Lower surface		Both surfaces		Upper surface		Lower surface		Upper surface		Lower surface	
			Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]	Actual water loss	Transpiration difference [2]
Standardizing period	Cloudy	126.1	134.9		136.7		131.3				129.4		136.4		130.0		129.4	
Correction applied			−8.8		−10.6		−5.2				−3.3		−10.3		−3.9		−3.3	
May 1	Fair	269.7	309.2	30.7	304.8	24.5	291.0	16.1			269.5	−3.5	295.4	15.4	251.4	−22.2	277.3	4.3
May 2	Cloudy	271.0	294.2	14.4	283.2	1.6	281.6	5.4			276.1	1.8	271.9	−9.4	247.0	−27.9	256.4	−17.9
May 3	do	373.3	409.6	27.5	376.1	−7.8	377.0	−1.5			353.4	−23.2	339.8	−43.8	327.0	−50.2	325.5	−51.1
Average		304.7	337.7	24.2	321.3	6.1	316.5	6.7			299.7	−8.3	302.4	−12.6	275.1	−33.4	286.4	−21.7
Corrected transpiration ratio			107.9		102.0		102.2				97.3		95.9		89.0		92.9	

EXPERIMENT NO. 9. SPRAYS APPLIED ON BRIGHT DAY JUNE 2, 1936: BORDEAUX 6-6-50; RED CUPROUS OXIDE 4-50. SODIUM LAURYL SULPHATE SPREADER IN ALL SPRAYS

Standardizing period	Cloudy	279.3	280.3		283.2		285.1		265.8		275.8		274.3					
Correction applied			−1.0		−3.9		−5.8		13.5		3.5		5.0					
June 2	Fair	540.6	641.9	100.3	616.6	72.1	539.7	−6.7	442.3	−84.8	498.1	−39.0	461.8	−73.8				
June 3	Cloudy	214.5	286.6	71.1	260.0	41.6	224.9	4.6	194.2	−6.8	210.9	−.1	202.8	−6.7				
June 4	Fair	233.3	288.0	53.7	277.2	40.0	255.2	16.1	213.1	−6.7	231.6	1.8	230.4	2.1				
June 5	do	281.3	312.2	29.9	323.8	38.6	298.1	11.0	261.5	−6.3	281.2	3.4	280.7	4.4				
June 6	do	274.8	288.4	12.6	313.6	34.9	279.9	−.7	262.0	+.7	268.4	−7.1	274.4	4.6				
June 7	Cloudy	194.2	178.0	−17.2	213.4	15.3	194.0	−6.0	195.0	14.3	204.0	13.3	200.9	11.7				
Average		289.8	332.5	41.7	330.2	40.4	298.6	3.0	261.4	−14.9	282.4	−6.1	275.2	9.6				
Corrected transpiration ratio			114.4		112.6		101.0		94.9		98.7		96.7					

[1] Water loss read at 8 a. m. Readings given were taken the day after.
[2] Transpiration difference is sprayed minus nonsprayed corrected for differences observed during standardizing period.

In four experiments the spraying days were bright days and the sun shone directly on the leaves (experiments 4 and 7, table 1, and experiments 8 and 9, table 2). During the 6 days following spraying bordeaux increased the daily average transpiration by 40.3, 38.4, 24.2, and 41.7 mg per square centimeter, respectively. In one experiment (experiment 6, table 1) the spraying day was dull, and bordeaux increased the daily average transpiration rate by only 11.1 mg per square centimeter. It follows, then, that high temperature while the leaves are wet with spray gives a higher bordeaux transpiration than does low temperature under similar conditions.

With reference to the weather that follows spraying, the experiments where sprays were applied on bright days are of interest. For example, in the February experiment (experiment 4, table 1) when cool dull weather followed spraying, the transpiration ratio was 138.6, whereas in the June experiment (experiment 9, table 2) when bright warm weather followed the spraying, the transpiration ratio was 114.4. These findings agree with those of Wilson and Runnels (*19*) who concluded on the basis of transpiration ratios that bordeaux gives a greater acceleration over the checks on dull days than on bright days.

However, it is interesting to examine the same data further by using transpiration differences instead of transpiration ratios. In the February test, the daily average transpiration difference of bordeaux was 40.3 mg per square centimeter, and in the June experiment, it was 41.7 mg per square centimeter. This means that 1 square centimeter of bean leaf surface sprayed with bordeaux lost about 40.0 mg more water daily than a nonsprayed leaf surface—irrespective of the brightness of the day and its effect on total water loss.

It follows that temperature while the spray is being applied seems to be important, but that temperature on succeeding days seems to have no differential effect on bordeaux transpiration.

EFFECT OF TIME

The transpiration differences for bordeaux in the various tests have been plotted against time (fig. 1). The curves show that the transpiration effect increases sharply the first day, and falls just as sharply the second day. In three of the five tests it increased again on the third day, but not so much as on the first day, and again fell off. In one test the secondary rise occurred on the fourth and fifth days followed by a decrease. The third peak in the curve for experiment No. 6 was caused by a second application. In only one test of the five was the secondary rise not observed.

EFFECT OF VARIOUS ELEMENTS IN BORDEAUX MIXTURE

Since bordeaux is a mixture and not a simple compound, its action must be studied by studying the action of its various constituents. Data were accumulated on the effect of the water, the copper, the lime, and a clay. An investigation was then made of the effects of certain qualities of the complete mixture such as hydrogen-ion concentration, filming and coverage, spray load, and capillarity of the film.

Effect of Water

Water is certainly an important element in bordeaux mixture. It is the water that distinguishes bordeaux from copper-lime dust.

It has already been shown that temperature at the time of spraying is important in bordeaux transpiration. The reason is that it is the

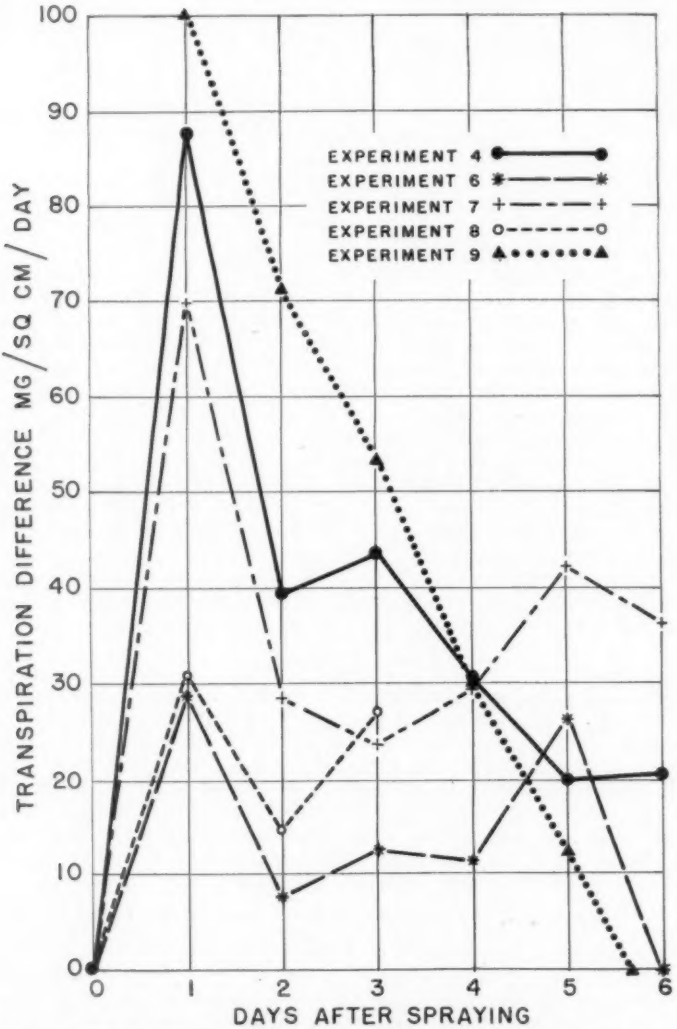

FIGURE 1.—Effect of bordeaux mixture on transpiration of beans on the first to sixth days after spraying. Data taken from tables 1 and 2.

only time (in the greenhouse at least) when water is on the leaf, and when the chemical reactions proceed that open the leaf for the escape of water.

In these tests the temperature on days following spraying was not important. The reason is that no water was present to permit further chemical reactions to proceed.

If water does play a role in producing bordeaux transpiration, dust applied to a dry leaf should produce little or no increase in water loss. Indeed such has been shown to be the case by Wilson and Runnels (*20*) and by Martin (*9*). It has been found at this station [6] that dusts give much less stunting of cucurbits than do sprays of the same materials.

Effect of Copper

Since the bean and tomato plants used here are not subject to copper flecking, the data obtained were limited to the effect of copper on lime-sensitive plants. The same can be said for essentially all transpiration data on sprays to date.

In the experiments with beans, an insoluble salt, red cuprous oxide, increased transpiration but little or not at all (experiments 4 and 7, table 1, experiment 8, table 2) when used at 1–50, equivalent in copper content to 4–4–50 bordeaux. The same can be said for four times the copper content or 4–50 (experiment 9, table 2). This failure to increase transpiration may possibly be caused by insufficient spray load [7] discussed later.

A soluble copper salt like copper sulphate is, of course, quite destructive to plants. This salt will kill the tissue in leaves of a plant like the tomato that is not considered sensitive to copper in bordeaux. When it kills the tissue it should give a wound acceleration of transpiration, followed by a rapid fall in the rate of transpiration as the tissue dies. Such has been shown to be the case when insufficiently neutralized bordeaux mixture is used (*24*). This reaction probably is not significant in bordeaux transpiration, however, because properly prepared bordeaux mixture contains no free copper sulphate.

Effect of Lime

On the other hand, most bordeauxs do contain some free lime. Bordeaux mixtures without excess of lime can be prepared, but since the system is unbuffered, a small quantity of excess lime makes the system quite alkaline.[8] From the standpoint of using bordeaux in practice an excess-lime formula must be recommended to the farmer since commercial limes may become partially carbonated before use. A study, therefore, of the effect of lime is pertinent to a study of bordeaux transpiration.

Hydrated lime alone increased transpiration markedly although it was not so effective as bordeaux. Likewise, lime showed a devastating effect on tomato transplants. Tomato seedlings were sprayed with various materials and transplanted next day to reasonably moist soil but not watered for 4 days (fig. 2). The plants sprayed with 4–4–50 bordeaux and an equal load of lime (8–50) failed to recover from the transplanting shock. As discussed later the difference between the effect of lime and the effect of bordeaux is complicated by the different filming of the two sprays.

[6] See footnote 5.

[7] The term "spray load" is used to mean the amount of material retained on the leaf. This, of course, is very difficult to measure. For the purposes of this discussion it is assumed that all sprays are applied and retained in an equal volume in any test. If so, spray load becomes a function of the amount of material per 50 gallons of spray and is so expressed.

[8] See footnote 5.

Carbonation of the hydrated lime reduced the transpiration effect. A lime sample containing 38 percent [9] of calcium carbonate was burned in a muffle furnace. This was then hydrated and compared on the plant with the original carbonated sample (experiments 6 and 7, table 1). When the sprays were applied on a bright day (experiment 7, table 1), the transpiration difference was 25.1 mg per square centimeter per day for the pure lime but only 5.4 mg per square centimeter per day for the carbonated lime. When applied on a dull day (experiment 6, table 1) the pure lime gave a transpiration difference of 5.9 mg per square centimeter per day, whereas the carbonated lime reduced the water loss by 5.3 mg per square centimeter per day. Wilson and Runnels (*18*) report a similar effect of carbonation.

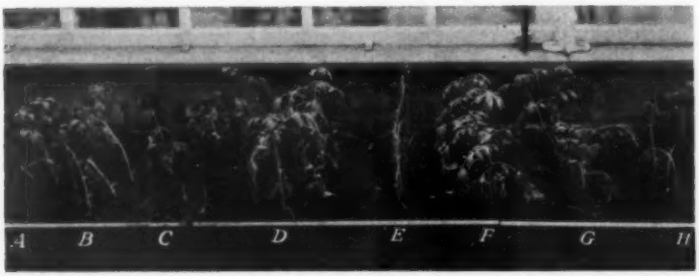

FIGURE 2.—Wilting effect of various sprays applied to tomato seedlings 1 day before transplanting: *A*, Nonsprayed; *B*, bordeaux 4–4–50; *C*, red cuprous oxide 1–50; *D*, bordeaux-cottonseed oil 4–4–½ percent–50; *E*, lime 8–50; *F*, red cuprous oxide-lime 1–4–50; *G*, nonsprayed; *H*, bordeaux 4–4–50.

Since hydrated lime alone caused accelerated transpiration and since reduction of the alkalinity by carbonation reduced the effect, it follows that the alkalinity of bordeaux probably is a significant factor.

There are other data on the effect of alkalinity on bordeaux transpiration. Wilson and Runnels (*20*) found that 4–6–50 copper acetate-lime increased transpiration more than did 1–50 copper acetate minus lime. They attributed this difference to the increased copper content of the former mixture. The presence of lime in the former mixture, however, may well be the cause. Basic copper sulphate (sometimes thought of as bordeaux minus lime) gave them very much smaller water losses than the usual 4–4–50 formula. They (*23*) found also that the addition of lime to liquid lime-sulphur (1–4–50) increased transpiration markedly over liquid lime-sulphur alone (1–50).

EFFECT OF CLAY

Inasmuch as alkalinity was found to be associated with bordeaux transpiration, the effect of an acid clay was investigated. Bancroft clay was the acid material tested (experiments 6 and 7, table 1). This clay, which is sometimes used as a diluent for fungicides and insecticides, is manufactured by United Clay Mines, of Trenton,

[9] This analysis was made through the courtesy of Prof. A. W. Clark of this station.

N. J. The transpiration rate was increased to about the same degree by Bancroft clay as by hydrated lime. In the first test (experiment 6, table 1) when the spray was applied on a dull day the transpiration differences were 0.0 and 5.9, respectively. In the second test, when the sprays were applied on a bright day, the transpiration differences increased to 26.9 and 25.1, respectively. The addition of red cuprous oxide to the clay had little or no effect on the transpiration difference. Wilson and Runnels (*25*) point out deleterious effects of clays on transplanting losses of tomatoes.

It seems to follow, then, that both acids and alkalis accelerate transpiration of beans.

Effect of Hydrogen-ion Concentration

The gross aspects of alkalinity and acidity on transpiration having been observed, it was of interest to relate the subject more clearly to hydrogen-ion concentration. The hydrogen-ion concentration of lime-water is approximately 12.3 and that of a Bancroft-clay suspension is about 3.5. These two materials serve to establish the ends of a curve for the effect of hydrogen-ion concentration on transpiration. Since they both accelerate transpiration, it seems clear that a point somewhere between is necessary to establish the low point of the curve. Theoretically the hydrogen-ion concentration of a spray mixture having little or no effect on transpiration should serve to establish the approximate location of this midpoint.

Since red cuprous oxide was known to give little or no acceleration of transpiration, the hydrogen-ion concentration of the suspension of this material was ascertained and was found to be 6.4. The effect of hydrogen-ion concentration of spray suspension on transpiration apparently follows a V-shaped curve with the bottom of the trough somewhere near pH 7.

In studying the stunting effects of bordeaux on cucurbits, Horsfall, Hervey, and Suit [10] have found that the least stunting is obtained with spray mixtures having a hydrogen-ion concentration somewhere near 7.

Wilson and Runnels (*12*; *18, pp. 175–179; 24*) have published data (assembled in table 3) on the effect on transpiration of varying the copper and lime ingredients in bordeaux. Among other effects, varying the copper and lime ratios in bordeaux varies the hydrogen-ion concentration of the mixture. The hydrogen-ion concentration of the mixtures they used has been determined. Unfortunately, their mixtures serve to establish only the acid and the alkaline end of the pH curve. They did not make any bordeaux mixtures with neutral hydrogen-ion concentration. Assuming that the transpiration of the nonsprayed leaves was the same as if they had been sprayed with a neutral material as in the present writers' experiments with red cuprous oxide, the curves from these data are also V-shaped. The data in table 3 show some variability of the points on the two ends of the pH scale. The cause of this variability is chiefly variation in spray load as discussed later. In the case of the high copper mixtures, there is also difficulty with soluble copper discussed above.

[10] See footnote 5.

TABLE 3.—*Relation of lime and copper variations, spray load, pH value and settling rate on transpiration and desiccation*

[Data assembled from Wilson and Runnels]

Bordeaux formula	pH [1]	Spray load per 50 gallons	Settling [2] rate, height after 1 hour	Transpiration [3] of coleus		Tomato transplants injured [3]	
				October	April	High Ca	High Mg
		Pounds	*Millimeters*	*Grams*	*Grams*	*Percent*	*Percent*
Nonsprayed				70	100	0	0
15-3-50	4.18	18	9.5	61	91	83	80
12-3-50	4.33	15	7.5	73	168	58	52
9-3-50	4.74	12	3.0	77	176	20	32
6-3-50	11.70	9	0.5	85	186	6	0
3-3-50	15.30	6	2.0	95	199	13	7
3-6-50	12.30	9	6.0	101	202	20	13
3-9-50	12.30	12	10.0	95	187	42	22
3-12-50	12.30	15	13.0	91	183	70	62
3-15-50	12.30	18	15.0	86	172	93	87

Bordeaux formula	pH	Spray load per 50 gallons	Desiccation		Transpiration [3]	
			Coleus [3]	Tomato, [4] percent of check	Coleus	Tomato
		Pounds	*Grams*	*Percent*	*Grams*	*Grams*
Nonsprayed			22			128
4-0-50	4.09	4	28	14		
4-½-50	4.36	4½	36	30		
4-1-50	4.36	5	41	41		
4-2-50	11.70	6	45	50		169
4-4-50	12.30	8	50	67		184
4-6-50	12.30	10	51	92		201
4-8-50	12.30	12	49	92		
4-9-50	12.30	13				206
4-12-50	12.30	16	46	63		207
Nonsprayed			25		52	78
0-6-50	12.30	6	29	16		
¼-6-50	12.30	6½		22		
1-6-50	12.30	7	32	28		
2-6-50	12.30	8	38	52	106	158
3-6-50	12.30	9	40	60		
4-6-50	12.30	10	47	88	110	162
6-6-50	12.30	12	46	90	102	152
9-6-50	12.30	15	48			
12-6-50	12.30	18	48			

[1] pH-value determined by George Pearce of this station.
[2] Reference (*18, pp. 175–179*).
[3] Reference (*24*).
[4] Reference (*12*).

EFFECT OF FILMING AND COVERAGE

It is recognized immediately that isolating the various chemical components of bordeaux merely gives indications of their functions. Completed bordeaux certainly has physical qualities not possessed by any of the chemical components. The colloidal, gelatinous, filming quality is peculiar.

Lime alone has seldom given quite as much transpiration as the completed bordeaux mixture, even when equivalent copper has been added to it in some so-called insoluble form, and has never given quite as much transpiration when combined with other metallic sulphates (*22*) as when combined with copper sulphate.

It is probable that this is a question of the superior contact which properly prepared bordeaux mixture makes with the leaf. The colloidal bordeaux film obviously makes closer contact with the leaf than do discrete particles of lime. To prepare the best bordeaux films

it is commonly suggested to pour the copper sulphate into the lime water rather than the reverse. Such procedure gave Runnels and Wilson (*12*) 70 and 54 percent, respectively, increase in water loss.

The experiment already described showing increased bordeaux transpiration with decreased carbonation of lime is related to filming as well as to increased alkalinity because hydrated lime gives a smoother film than carbonated lime.

Bordeaux loses much of its filming effect upon standing. Wilson and Runnels (*21*) show that 4-day-old bordeaux is much less effective in accelerating transpiration than new bordeaux. The finding (*20*) that soluble salts plus lime give larger transpirational effects than insoluble salts plus lime is probably due to the better filming of the former.

Effect of Spray Load

In the test on transplants shown in figure 2, 8–50 lime produced more injury than 4–50 lime. This probably is a question of spray load, because the hydrogen-ion concentration of these mixtures is the same. It is true, however, that the 4–50 lime carried also 1 pound of red cuprous oxide, but this probably did not reduce the transpiration. Although the difference is small, Duggar and Cooley (*3*) found more transpiration from 4–6–50 than from 2–3–50 bordeaux, probably because of the difference in spray load.

The experiments of Wilson and Runnels (*12; 18, pp. 175–179; 24*) varying the copper and lime contents of bordeaux mixture are very illuminating from the standpoint of spray load. Obviously as either component is increased while the other remains constant, the spray load also increases. Water loss was measured in three ways: (1) Transpiration from potted plants, (2) desiccation of cut shoots, and (3) injury to transplants. Pertinent data from these authors are arranged in table 3 and as curves in figures 3 and 4. Generally speaking, desiccation and injury to transplants increased with spray load irrespective of whether it was shifted by varying the copper or the lime. Transpiration, on the other hand, increased with the spray load only to a point of about 2 parts of lime to 1 of copper and then decreased sharply.

Possible reasons for the discrepancy between desiccation and transpiration experiments are given in the section on the effect of leaf surface.

It follows that spray load on the leaf is closely correlated with water loss and should be considered in experiments where bordeaux formulas are varied.

Effect of Capillarity of Bordeaux Film

The assumption (*2*) that bordeaux films dissipate water by something akin to capillarity over the large surface of spray film exposed fits the data and helps to explain the effect of filming and spray load.

Effect of Oil

Oil emulsions are known to facilitate filming and coverage of sprays, but it is common knowledge (*17*) that oils generally decrease rather than increase the transpiration rate of plants whether sprayed with bordeaux mixture or not.

Cottonseed-oil emulsion was used as a spreading and coverage agent (experiment 4, table 1; and experiment 8, table 2, fig. 2).

This material alone or in combination with red cuprous oxide reduced the transpiration rate below that of nonsprayed leaves. On first thought this result may be taken as evidence that the oil film bridges over the stomata and curtails water loss from them. On the other hand there is evidence that cottonseed oil does not materially affect

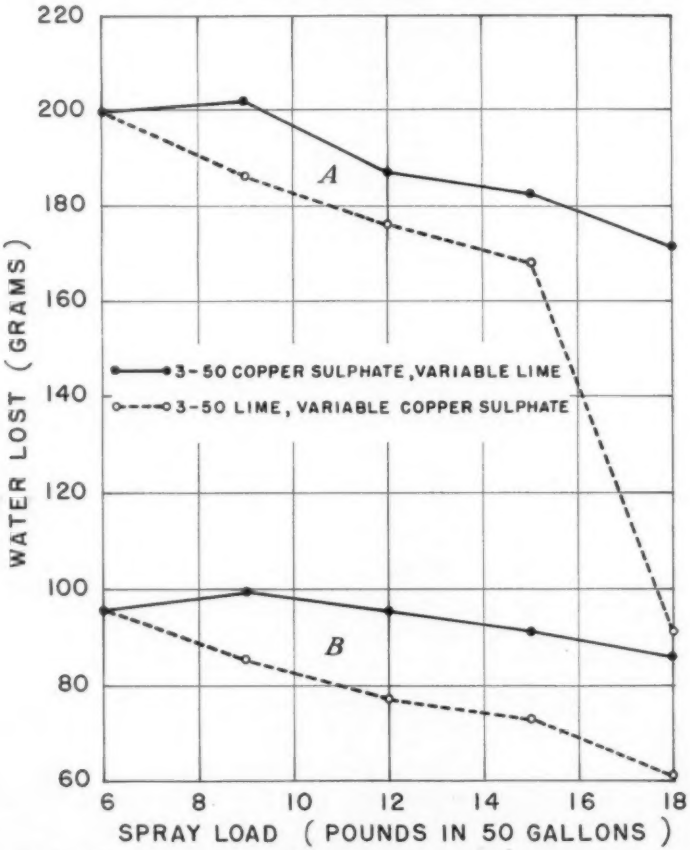

FIGURE 3.—Effect of spray load of bordeaux mixture on transpiration of coleus; data from table 3: *A*, Transpiration in April; *B*, transpiration in October.

stomatal transpiration. In one experiment (experiment 8, table 2) bean leaves sprayed on the lower side (where most of the stomata are) lost more water, if anything, than did bean leaves sprayed on the upper side, although both lost less than the nonsprayed leaves.

Two possibilities for explaining the action of the oil alone in reducing transpiration are (1) that it seals abrasions in the cuticle, and (2) that it functions to make a thicker oily barrier to water loss than the cuticle alone provides.

The reduction in transpiration caused by adding oil to bordeaux may be due to a saturation of the film with oil so that water cannot

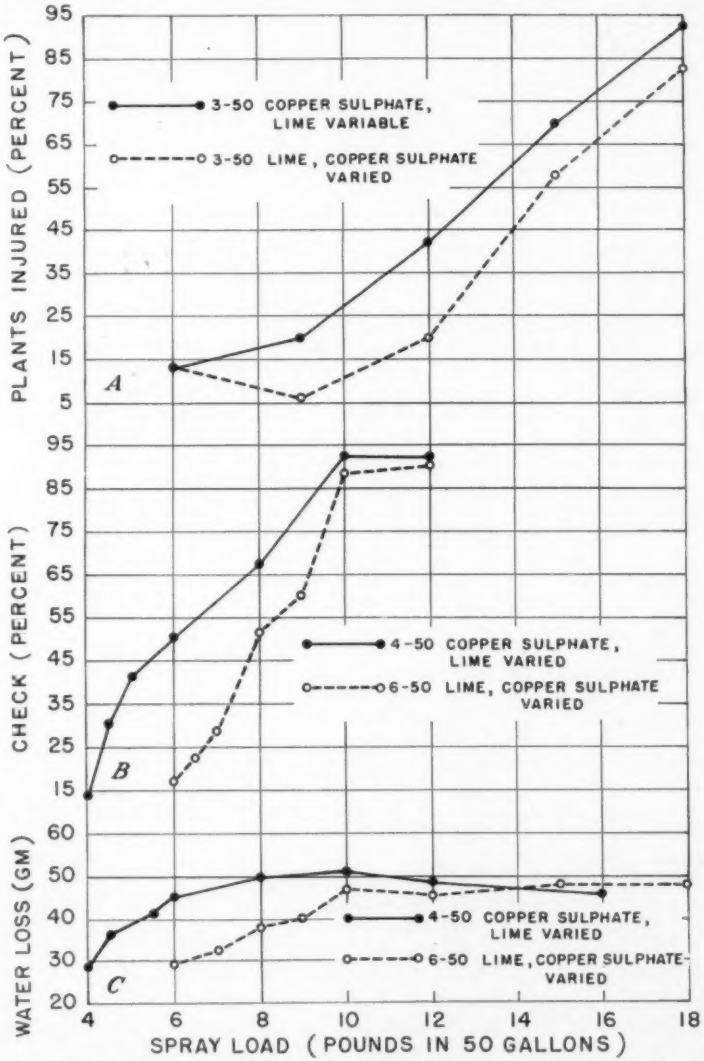

FIGURE 4.—Effect of spray load on desiccation of coleus and tomatoes, and injury to tomato transplants; data from table 3: *A*, Injury to tomato transplants; *B*, desiccation of tomatoes; *C*, desiccation of coleus.

dissipate itself so readily by capillarity. This effect would be in addition to the basic effect of the oil in the absence of bordeaux.

EFFECT OF LEAF SURFACE

Normal leaves give off water primarily through the stomata, although there appears to be some loss directly through the cuticle. In studying bordeaux transpiration, the question arises as to whether only one or both systems are affected and to what extent. Bordeaux might affect transpiration in four possible ways: (1) Increase it by propping the stomata open (*2*) and permitting water to escape, (2) decrease it by clogging the stomata as suggested by Clinton (*1*), (3) increase it by lowering cuticular resistance to water loss, and (4) decrease it by cooling the leaf as shown by Tilford and May (*15*).

Effect on the Stomata

The opening or closure effects of bordeaux on stomata have been studied by spraying bean plants on the upper leaf surface where stomata are practically absent, and separately on the lower leaf surface where they are present. If sprays do not affect the stomata, transpiration should be the same whether the material is applied to the upper or to the lower leaf surface. If the spray props the stomata open, the transpiration of leaves sprayed below should be greater than that of leaves sprayed above, because the stomata would then remain open at night and lose water when they would normally be closed. If, however, the spray clogs the stomata, the transpiration of leaves sprayed below should be less than that of leaves sprayed above, because the stomata would lose less water than normal in the daytime.

The latter proved to be the case. In two tests (experiments 8 and 9, table 2) leaves sprayed below with bordeaux transpired less than those sprayed above. Apparently the spray clogged the stomata and did not prop them open. The magnitude of the plugging effect can be calculated with some degree of accuracy. The transpiration difference cancels out the normal effects of stomata and cuticle on nonsprayed leaves. Let the transpiration difference of leaves sprayed above be the cuticular effect of bordeaux and of those sprayed below be the resultant of the effect on lower cuticle and plugging effect on stomata. In the first test (experiment 8, table 2) the transpiration difference on the first day after spraying the upper surface was 24.5 mg per square centimeter per day. The sprayed lower surface should have transpired as much as the upper if no stomatal action were involved, but actually the transpiration difference was only 16.1 mg per square centimeter per day. The 34 percent smaller transpiration of the lower surface than the upper indicates the degree of stomatal plugging. The stomata transpired that much less than normal.

In the second test (experiment 9, table 2) the difference was still larger than in the first test perhaps because of better coverage. In this test the sprayed upper surface transpired 72.1 mg per square centimeter per day more than the nonsprayed on the first day after spraying, while the sprayed lower surface transpired 6.7 mg per square centimeter less than the nonsprayed. In this test the spray on the lower surface plugged enough stomata to eliminate the accelerating effect of the spray on the cuticle.

The magnitude of the stomatal plugging with bordeaux spray can be calculated also from some data of Wilson and Runnels (*16*) who used coleus, which, like the bean, has most of the stomata below. At night when the stomata were closed, the nonsprayed leaves lost 1.09 g of

water per hour. Leaves sprayed above lost 2.68 g per hour, and those sprayed below lost 4.66 g per hour. In the daytime, on the other hand, between 10 a. m. and 1 p. m., when stomata are open widest, the nonsprayed leaves lost 17.7 g per hour, those sprayed above lost 17.9 g per hour, and those sprayed below lost 16.5 g per hour.

The magnitude of the plugging effect can be calculated as follows: Let normal cuticular transpiration be the water loss of the nonsprayed leaves at night (1.09 g per hour). Let the normal stomatal transpiration be the water loss of the nonsprayed leaves in the daytime less the normal cuticular transpiration (17.70−1.09=16.61 g per hour). Let the bordeaux effect on the lower cuticle be the water loss of the lower-sprayed leaves at night minus the normal cuticular transpiration (4.66−1.09=3.57 g per hour).

If the bordeaux sprayed below has no effect on stomata, the daytime theoretical transpiration of the lower sprayed leaves should be the normal stomatal transpiration plus the bordeaux effect (16.61+3.57=20.18 g per hour). The actual transpiration of the lower sprayed leaves, however, was 15.41 g per hour (16.50−1.09). This gives a reduction from the theoretical of 4.77 g per hour, or 23.6 percent, which represents for this test the magnitude of the effect of stomatal plugging.

Effect on Cuticular Resistance to Water Movement

Since the accelerating effect of bordeaux cannot be due to an effect on stomata, it must be an effect on the cuticle, as first proposed by Wilson and Runnels (*16*). The other facts that point to the cuticle are (1) bordeaux increases transpiration at night when stomata are closed, and (2) a spray on the upper leaf surface where stomata are scarce increases transpiration.

It follows, then, that bordeaux mixture has a two-way action on transpiration—it accelerates cuticular transpiration and curtails stomatal transpiration.

The discrepancy in results between desiccation of cut shoots and transpiration of potted plants, discussed above, as regards spray load is interesting from the standpoint of cuticular versus stomatal transpiration. In experiments on desiccation of detached foliage the stomata probably become closed on account of wilting at the very outset of the test. In such a case only the cuticular transpiration is measured. If so, the water loss should increase as it does increase with the spray load because of (1) more complete coverage and (2) increased capillary surface exposed to evaporation by the spray films.

Where total transpiration is measured on potted plants, however, the record represents a summation of cuticular and stomatal transpiration, both of which proceed together. Presumably, increasing the spray load should increase cuticular transpiration both on cut shoots and on potted plants. Increasing the spray load should give decreasing stomatal transpiration, however, so that in the case of potted plants the measurable transpiration should be a resultant of the operation of these two factors. Since the transpiration curve for potted plants generally goes down as the spray load goes up, it is probable that the reduction in stomatal transpiration with increasing spray load proceeds more rapidly than the increase in cuticular transpiration.

Bordeaux should produce less effect on thick than on thin cuticle. That is probably why in the tests bordeaux applied to the lower leaf surface caused more wilting than when applied to the upper, and why (*16*) lower sprayed leaves lost more water at night than upper sprayed leaves (4.66 as compared with 2.68 g per hour). Generally the upper leaf surface in the tests recovered faster than the lower leaf surface (table 2) from the initial transpiration peak, presumably because the thick upper cuticle repaired itself faster than did the thin lower cuticle. If the lower cuticle is more subject to bordeaux damage than the upper, the stomatal plugging effects discussed above are really larger than calculated, because the calculations are based on the assumption that the cuticular effect is the same on both surfaces.

Practically all research to date on bordeaux transpiration has been done in the greenhouse in the North in the wintertime when light intensity is low and where presumably cuticles would be thin. It seems significant that the careful work of Miller (*11*) during 4 years on large tomato vines growing in the open under relatively dry conditions of Kansas showed no significant acceleration of bordeaux transpiration. Miller was probably dealing with a thicker cuticle than that usually found in greenhouses in winter.

On the basis of these findings, it is probable that bordeaux at times may actually reduce transpiration. Such an effect could be produced (1) when cuticular loss is low because of thickened or hardened cuticle and (2) when stomatal plugging is paramount.

Effect on Leaf Temperature

Wilson and Runnels (*16*) found the sprayed upper surface of leaves also lost less water than the nonsprayed leaves in the middle of the day, when on the basis of the nighttime results they should have lost more. Wilson and Runnels ascribe this to a cooling effect of the spray film on the upper leaf surface. In the case of Miller's experiments, if spray below clogs the stomata and spray above cools the leaf, the total of the two effects may have equaled the cuticular loss obtained.

SUGGESTED HYPOTHESIS FOR BORDEAUX TRANSPIRATION

It seems superfluous to conclude that bordeaux may increase transpiration. The question is rather how does bordeaux increase transpiration. An exit must first be provided for the water. The usual channels, the stomata, seem ruled out (*7*). They appear to be plugged, if anything. The cuticle must, therefore, be opened for the passage of water outward, or perhaps more accurately, its resistance to the passage of water outward must be decreased.

It seems reasonably clear that the lime factor, or rather the alkalinity of the lime factor, is very important if not chiefly responsible for the bordeaux water loss, although acid sprays also seem to increase transpiration, whereas neutral sprays do not. Miller (*11*) says that two of the characteristics of plant cuticle are that it is readily soluble in warm alkalis and readily oxidized by certain acids. The spray material (note larger transpiration from sprays than from dusts) supplies the liquid. A high temperature at the time of spraying provides the warmth and the lime supplies the alkali (in bordeaux). Once the cuticle has been partially saponified by the alkali, it is probable that water can pass outward more readily than before. The bordeaux

effect rapidly diminishes (fig. 1). The lime probably carbonates, as shown by Streeter, Mader, and Kokoski (*14*), and is no longer sufficiently alkaline; in any case the spray has dried, so that further saponification cannot proceed. New fats move up from the inner leaf and gradually repair the broken cuticle in 3 or 4 days so that the transpiration effect dwindles.

The acid sprays probably act in much the same manner except that the acids oxidize instead of saponify the cuticle. In either case the resistance of the cuticle to the passage of water is diminished.

It does not seem so certain now that bordeaux will always increase transpiration or that the experiments of Miller (*11*) and Lutman (*8*) can be disregarded. These men worked with mature plants in the field where the plant cuticle is usually thick and hard. The accelerated transpiration effects have been observed chiefly in the greenhouse where the cuticle is usually thin and soft.

It is well within the bounds of reason that if the cuticle is so hard as to resist saponification by alkaline sprays, the lessening effects of bordeaux may overshadow the accelerating ones, so that the summation measured might show even a reduced, not an accelerated, effect.

SUMMARY AND CONCLUSIONS

The experimental work on the effect of bordeaux mixture on transpiration of tomato and bean plants was done in the greenhouse in the wintertime.

Transpiration was measured with autoirrigators and expressed on unit-area basis. The effect of sprays is expressed as transpiration differences or as transpiration ratios.

On the basis of this research and facts available in the literature the following conclusions are reached:

High temperature on spray date favored bordeaux transpiration. High temperature after spray date had little effect.

Of the elements in bordeaux, the lime or rather the lime-induced alkalinity seemed to be the most important, although unneutralized copper sulphate is certainly quite destructive to the leaves.

Any method of making the copper insoluble, even by using it as a dry dust, almost eliminated any of its transpirational effects.

Bancroft clay, a so-called inert material, had an accelerating effect on transpiration presumably because it was acid.

Increase in coverage or filming qualities of bordeaux increased transpiration effects.

Increasing spray load accelerated desiccation of cut plants. On potted plants an increase in spray load increased transpiration to a point beyond which a further increase in spray load decreased transpiration.

Cottonseed-oil emulsion reduced transpiration effects.

Bordeaux decreased cuticular resistance to water loss.

Bordeaux may clog stomata, rather than prop them open, and may increase water loss by capillarity over the surface of the spray film.

A tentative hypothesis of bordeaux-induced transpiration is this: The alkalinity of bordeaux saponifies the cuticle and decreases its resistance to water loss. At the same time, however, it may clog some stomata; it may cool the leaf in bright weather. The balance between these various factors will determine the magnitude of bor-

deaux transpiration. In the field, where cuticles are thick and hard, it may be true at times that the effect on the cuticle may be so slight as to be overbalanced by the factors for reduced water loss. This point needs further research.

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MORPHOLOGY OF TWO AMERICAN WILD SPECIES OF COTTON AND OF THEIR HYBRID[1]

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INTRODUCTION

Collections of living plants of numerous species of *Gossypium* are maintained by the Bureau of Plant Industry at Riverside and Palm Springs, Calif. Cytological study of these plants by J. M. Webber (*10*),[2] supporting conclusions reached by other investigators, has established the facts that, in all cultivated Asiatic forms and in all species in different parts of the world that have lintless seeds and are unquestionably indigenous in the localities where they occur, the haploid chromosome number is 13, and that, on the other hand, the cultivated American species and the wild or semiwild forms that are evidently related to them and have long lint hairs on the seeds have a haploid chromosome number of 26.[3]

Hybrids between many pairs of species of all three groups (wild lintless, cultivated Asiatic, and cultivated American cottons) have been made by J. M. Webber (*11*), but very few of these have proved fertile. The most outstanding instance of fertility is *Gossypium armourianum* × *G. thurberi*, a hybrid between two wild lintless North American species. F_2 populations, totaling 64 individuals, were grown in 1937 and 1938.

It is hoped that study of interspecies hybrids in the genus *Gossypium* will lead to a better understanding of relationships in this genus and eventually to a more satisfactory classification of the species, and that the puzzling question of the origin of the tetraploid cultivated American cottons may be answered. Realization of this hope seems to depend upon the possibility of inducing doubling of the chromosomes in some of these interspecies hybrids. There is also the possibility, remote, perhaps, but not to be ignored, that cottons superior in resistance to drought, diseases, and insect pests may be developed, if it should prove practicable to obtain fertile hybrids between wild species and some of the cultivated cottons.

The importance of interspecific hybridization in this genus makes it desirable to compare in some detail the morphological characters of *Gossypium armourianum* × *G. thurberi*, the first decidedly fertile hybrid between wild species, with those of the parental forms.

The natural ranges of the parent species do not overlap. *Gossypium armourianum* Kearney (*7, 8*) is known only from the island of San Marcos, in the Gulf of California (lat. 27° 15′ N.). *G. thurberi* Todaro (*Thurberia thespesioides* A. Gray) (*8*) occurs in the mountains of

[1] Received for publication November 7, 1938. The gross morphology was studied by T. H. Kearney, assisted by J. M. Webber in the classification of the F_2 population. The leaf anatomy was investigated by Irma E. Webber.

[2] Italic numbers in parentheses refer to Literature Cited, p. 459.

[3] Examples are *Gossypium tomentosum* Nutt., *G. taitense* Parl., and *G. darwinii* Watt. These species, which grow in the wild on islands in the Pacific Ocean, have well-developed though short lint hairs on the seeds and are clearly related to the American cultivated cottons.

southern Arizona and southward in Mexico to the State of Jalisco. The two species are so strikingly different in appearance and structure that fertility in the hybrid between them would scarcely have been expected, although the experience of hybridizers in other groups of plants has shown that morphological similarity is not necessarily an indication of cross-compatibility, and vice versa. Notwithstanding the great morphological dissimilarity of these species, J. M. Webber (*11*) found them to be very similar in cytological behavior.

GROSS MORPHOLOGY OF THE PARENT SPECIES AND F_1

Such of the contrasting characters of *Gossypium armourianum* and *G. thurberi* as were used in classifying the F_2 population and the expression of these characters in the first generation of the hybrid are stated

FIGURE 1.—Plant of *Gossypium armourianum* grown at Bard, Calif., from seed from San Marcos Island, Baja California.

in table 1, columns 1 to 4. The general appearance of the plants and details of some of the characters are shown in figures 1 to 5. Attention is directed particularly to the contrast between the small, round, entire leaf of *G. armourianum* (fig. 3, *A*) and the large, pedately lobed leaf of *G. thurberi* (fig. 3, *B*), which resembles that of the okra-leaf variation in upland cottons.

The hybrid (F_1) resembled *Gossypium armourianum* in its thick, somewhat shiny leaf blades, caducous bractlets (falling off long before anthesis), dentate calyx, white stigmas and brown oil glands of the pistil; also (characters not stated in table 1) in its glabrous leaf blades, very small extrafloral nectary on the midvein, relatively long pistil, and number of ovules per locule of the ovary. It resembled *G. thurberi* in the presence of large extrafloral nectaries on the peduncle and in the degree of lobation of the leaf blades; also (characters not stated in table 1) in the color of the older bark, whitish-margined leaf blades, and narrowed apex of the basal sinus of the blade. In the following

characters the hybrid was more or less intermediate: Shape of cross section of the twigs; character of the apex and length of the leaf blades; constriction of the midlobe at base; and length of the bractlets.

FIGURE 2.—Plant of *Gossypium thurberi* (*Thurberia thespesioides*), grown at Riverside, Calif., from seed from Arizona.

SEGREGATION OF CHARACTERS IN F_2

The classification of the F_2 population in respect to several of the characters that differentiate the parents is shown in table 1, columns 5, 6, and 7. The progenies of the two F_1 individuals (W8 and W9) are treated as one array in column 5, but the two progenies are also classi-

fied separately in columns 6 and 7, because they segregated differently in some of the characters. It will be noted that the total number of individuals is smaller in the classifications of flower characters than of

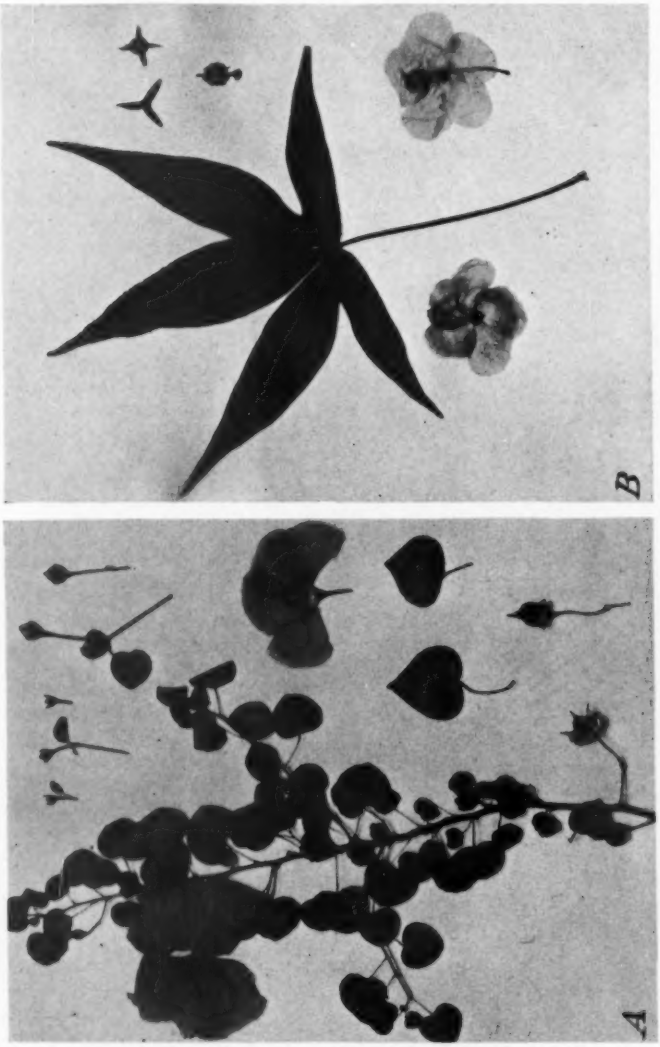


FIGURE 3.—Details of leaves and flowers of parent species, photographed from herbarium specimens, ⅓ natural size. *A*, *Gossypium armourianum*. Note the small, entire leaves and the very small bractlets that persist only while the flower bud is very small (three buds, upper center), dropping off while the bud is still small and long before the flower opens (two buds, upper right). *B*, *G. thurberi*. Note the relatively large, deeply lobed (pedate) leaf and the larger bractlets (upper right) which persist until the boll matures.

stem and leaf characters. This is because some of these F_2 plants failed to produce flowers.

The frequency distributions show no evidence of monohybrid segregation except in shape of the cross section of the twigs, in which

character there was a near approach to a 1:2:1 ratio, χ^2 of the departure (both progenies as one array) being 0.207. This small value for χ^2 indicates a high degree of probability that the departure of the observed from the expected ratio was fortuitous. On the other hand,

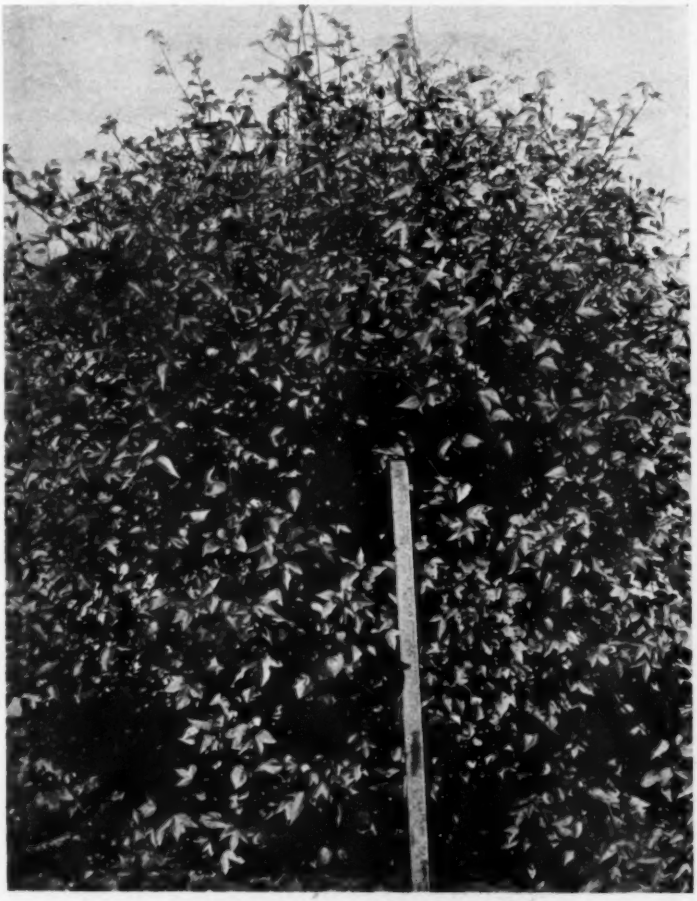


FIGURE 4.—First-generation plant of the interspecific hybrid *Gossypium armourianum* × *G. thurberi*, grown at Riverside, Calif. Note the intermediate condition in regard to compactness of branching and lobation of the leaves, as compared with the expression of these characters in the parent species (figs. 1 and 2).

the extreme rarity of monohybrid segregation in interspecific hybrids causes one to wonder if the classification was faulty. It should be stated, however, that every effort was made to have it objective and it was not suspected, when the plants were being classified, that definite segregation would appear.

FIGURE 5.—Second-generation plants of *Gossypium armourianum* × *G. thurberi*: *A*, A plant that approaches the *G. armourianum* parent in its relatively compact habit and small, very shallowly lobed leaves; *B*, a plant that approaches the *G. thurberi* parent in its open habit and large, deeply lobed (pedate) leaves.

TABLE 1.—*Contrasting characters of Gossypium armourianum and G. thurberi and expression of these characters in F_1 and segregation in respect to them in F_2 of the hybrid G. armourianum × G. thurberi*

Character and expression	*G. armouri-anum*	*G. thur-beri*	*G. armourianum × G. thurberi*			
			F_1	Classification of F_2		
				Both progenies as one array	Progeny W8	Progeny W9
Twigs, shape of cross section:						
Circular	×			15	8	7
Intermediate			×	30	17	13
Quadrangular		×		13	7	6
Leaf blade, thickness:						
Thick	×		×	24	12	12
Intermediate				30	17	13
Thin		×		5	3	2
Leaf blade, surface:						
Shiny	×		×	20	11	9
Intermediate				28	16	12
Dull		×		11	5	6
Leaf blade, lobation:						
None or very slight	×			14	10	4
Intermediate				17	9	8
Very deep		×	×	28	13	15
Leaf blade, apex:						
Obtuse or acutish	×			12	8	4
Short-acuminate			×	23	11	12
Attenuate-acuminate		×		24	13	11
Leaf blade, length:						
4 cm or less	×			19	11	8
5 to 9 cm			×	40	21	19
10 cm or more		×		0	0	0
Midlobe, basal constriction:						
None	×			32	21	11
Shallow			×	18	10	8
Deep		×		9	1	8
Peduncle, nectaries:						
None	×			22	16	6
Present but small				21	9	12
Present, large		×	×	14	6	8
Bractlets, persistence:						
Caducous when bud is very small	×		×	39	17	22
Less caducous				14	11	3
Persistent until anthesis		×		1	0	1
Bractlets, length:						
5 mm or shorter	×			45	21	24
5 to 9 mm		×	×	9	7	2
10 mm or longer		×		0	0	0
Calyx margin:						
Conspicuously dentate	×		×	35	19	16
Slightly dentate		×		16	9	7
Entire or merely undulate		×		3	0	3
Petal color:						
Pale yellow	×			23	6	17
Ochroleucous			×	16	11	5
White		×		13	9	4
Petal spot:						
Distinct	×	×		16	7	9
Very faint			×	13	5	8
None	×	×	×	23	14	9
Pollen color:						
Clear yellow	×			10	1	9
Pale yellow			×	12	1	11
Cream		×	×	29	24	5
Stigmas, color:						
White	×		×	31	14	17
Greenish white				15	10	5
Green		×		5	1	4
Stigmas, oil glands, color:						
Red brown	×		×	29	18	11
Yellowish				20	7	13
Green		×		2	0	2

In regard to certain characters in which F_1 resembled *Gossypium armourianum*, viz, thick leaf blades, shiny leaf surface, caducous bractlets, dentate calyx, white stigmas, and brown oil glands of the stigmas, F_2 plants that resembled *G. armourianum* were much more numerous than those that resembled *G. thurberi*. The marked dominance of deciduous bractlets (fig. 3, *A*) is noteworthy in view of the fact that in all cultivated cottons and in nearly all of the wild species they persist until the boll is mature. The bractlets in *G. thurberi* (fig. 3, *B*), although much smaller than in any cultivated cotton, are equally persistent.

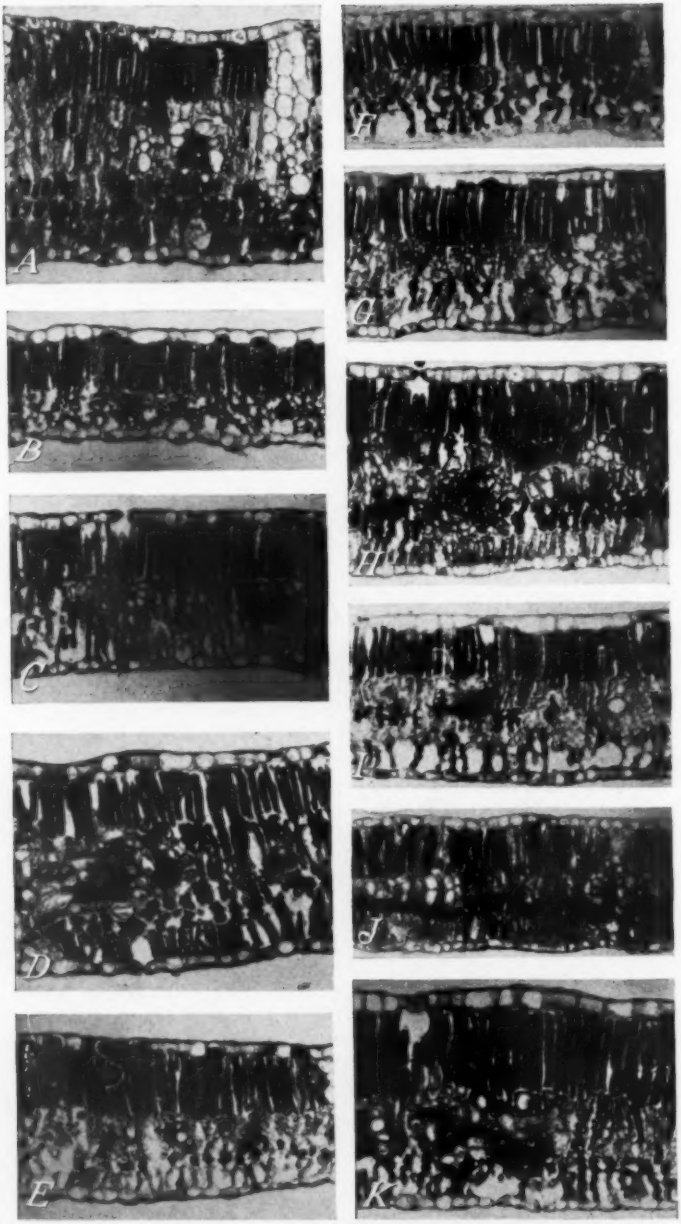
Characters in respect to which F_1 and a considerable majority of F_2 plants resembled *Gossypium thurberi* more closely than *G. armourianum* were pronounced lobation and acuminate apex of the leaf blades. No pronounced dominance of either parent is shown by the F_2 distribution for presence or absence of large extrafloral nectaries on the peduncle, although presence was dominant in F_1. The color of the petals was intermediate in F_1, but the F_2 population shows a tendency to dominance of the pale yellow of *G. armourianum*. In intraspecific crosses among cultivated cottons, yellow is almost completely dominant in F_1 and the segregations in F_2 indicate a ratio of 3 (full and paler) yellow to 1 white (*4*).

The evidence from the F_2 population, as well as from F_1, indicates that the number of dominant genes contributed by the *Gossypium armourianum* parent was larger than the number of dominant genes contributed by the *G. thurberi* parent.

It remains to consider the characters in respect to which the two F_2 progenies showed pronounced differences in their frequency distributions. These are leaf lobation, midlobe constriction, peduncle nectaries, petal color, petal spot, and pollen color. The F_1 parent individuals, W8 and W9, have not been observed to differ markedly in respect to the first four characters, so no explanation of the differences between the F_2 progenies is forthcoming at present. In view of the small size of the populations, the differences may not be significant.

As regards petal spot, there was segregation in F_1. Plant W8 had no spot on the petals of most of the flowers, whereas nearly all of the flowers on plant W9 had faint spots. The individual difference in petal spot in F_1 is accounted for by the fact that both *Gossypium armourianum* and *G. thurberi* are heterozygous in the wild, as well as in the populations grown at Riverside, for presence of a faint petal spot as contrasted with its absence. The *G. armourianum* parent of both W8 and W9 had spotless petals, but different individuals of *G. thurberi* served as parents of W8 and W9 and these were recorded as spotless and spotted, respectively. This probably explains the greater proportion of spotted flowers in F_2 progeny W9 (table 1). In view of the weak expression of this character in both parent species, classification by phenotypes is very uncertain and it is not unlikely that in reality all of the parents of the original crosses were heterozygous.

The F_1 plants W8 and W9 differed also in pollen color, the pollen of W8 being cream-colored and that of W9 being pale yellow. This segregation in F_1 is inexplicable, no appreciable variation in color of the pollen having been observed in the parent species. So far as has been observed, the pollen is always apricot yellow in *Gossypium armourianum* and uniformly cream-colored in *G. thurberi*. It will be noted



Cross sections of leaf blades of *Gossypium*, showing thickness and structure of the mesophyll, × 96: *A*, *G. armourianum*; *B*, *G. thurberi*; *C*, *G. armourianum* × *G. thurberi*, F_1, plant W9; *D*, the backcross (*G. armourianum* × *G. thurberi*, F_1) × *G. armourianum*, F_1; *E*, the backcross (*G. armourianum* × *G. thurberi*, F_1) × *G. thurberi*, F_1; *F*, W8, F_2, plant 2; *G*, W8, F_2, plant 28; *H*, W8, F_2, plant 12; *I*, W8, F_2, plant 14; *J*, W9, F_2, plant 9; *K*, W8, F_2, plant 25.

(table 1) that in the progeny of the F_1 plant that has cream-colored pollen (W8) a large majority of the F_2 individuals resemble the parent, whereas in the progeny of the F_1 plant with pale-yellow pollen (W9) the number of plants with yellow pollen exceeds the number with cream-colored pollen.

In intraspecific crosses among American cultivated cottons, presence of petal spot and yellow pollen are dominant to absence of spot and cream-colored pollen, respectively, with definite 3:1 segregation in F_2. Reference to table 1, columns 5 to 7, shows no indication that these small populations segregated in a definite ratio in respect to these characters; but, especially if the F_2 population as one array is considered (column 5), absence of petal spot and cream-colored pollen would appear to be imperfectly dominant. This is the reverse of what would be expected from the behavior of intraspecific crosses and of hybrids between 26-chromosome species, such as *Gossypium hirsutum*×*G. barbadense* (*2; 3; 5, pp. 22, 26; 6*).

LEAF ANATOMY OF THE PARENT SPECIES AND THEIR HYBRID

CHARACTERS INVESTIGATED, MATERIAL, AND METHODS

Little has been published hitherto on the gross morphology of hybrids involving American wild cottons, and there has been no report concerning the histology of such hybrids. There is much variation in the shape and size of the leaves on individual plants of *Gossypium thurberi*, but a previous histological survey of a number of species of *Gossypium* (*9*) showed that certain anatomical features of the leaf blade and petiole are fairly constant in this species. Epidermal characters are among such relatively constant features in *G. thurberi*, although differences in the pubescence and stomata of the leaves of this species and those of *G. armourianum* are quantitative rather than qualitative. On the other hand, *G. armourianum* and *G. thurberi* are characterized by striking differences in the structure of their mesophyll and petioles. This fact, coupled with the comparative difficulty of making and photographing preparations showing the epidermal surface, suggested that a comparison of the anatomy of hybrids between *G. armourianum* and *G. thurberi* might well begin with an examination of sections of their leaf blades and petioles.

All of the plants upon which this report is based were grown in a field at the Rubidoux Laboratory, Riverside, Calif. From each plant, fully matured leaves of typical vegetative shoots grown in exposed positions were chosen for study. Segments for sectioning were cut from approximately the center of the lamina and the middle of the petiole of each of the leaves. This material was killed in chrom-acetic formalin, dehydrated with *n*-butyl alcohol, embedded in paraffin, sectioned with a rotary microtome, stained with safranine, and mounted in balsam.

STRUCTURE OF THE MESOPHYLL

The Parent Species

The leaves of *Gossypium armourianum* (pl. 1, *A*) commonly have all of the chlorenchyma in the form of palisade tissue. Occasionally some of the chlorenchyma cells in the interior of the leaves are not greatly elongate and tend to be irregular in form, but typical well-developed palisade tissue always adjoins both the upper and the lower epidermis. Since as many as six layers of palisade cells are frequently

present in the leaves of *G. armourianum*, the mesophyll of this species is characteristically thick as well as dense.

In contrast to the leaves of *G. armourianum*, those of *G. thurberi* (pl. 1, *B*) are relatively thin and distinctly bifacial. Their palisade tissue is restricted to the layer of cells subjacent to the upper epidermis of the leaf. The remainder of the chlorenchyma is composed of several cell layers of rather loose spongy parenchyma.

First-Generation Hybrids and Backcrosses

The two plants of *Gossypium armourianum*×*G. thurberi* F_1 designated as W8 and W9 have the leaves intermediate between those of the parental species in respect to thickness and mesophyll structure (pl. 1, *C*). Their mesophyll is characterized by a well-developed layer of palisade parenchyma subjacent to the upper epidermis; a layer in part spongy parenchyma but predominantly weak palisade parenchyma, which adjoins the lower epidermis; and several intervening layers of more or less elongate cells.

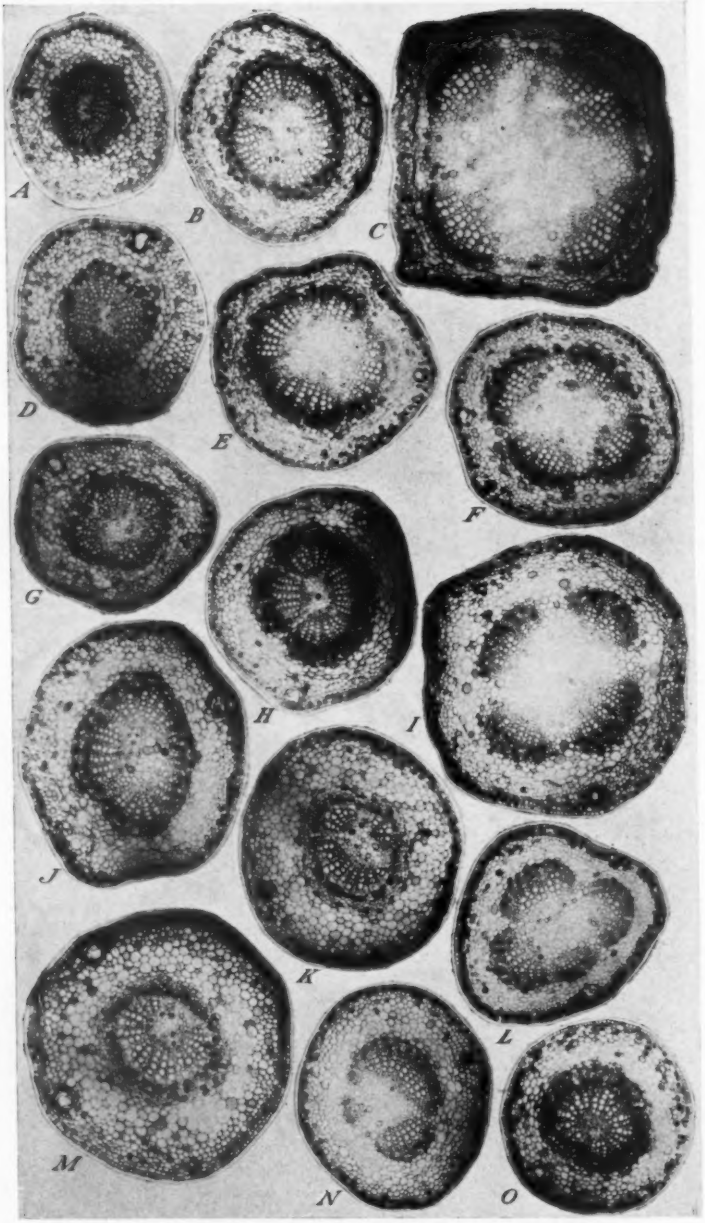
Mesophyll structure in a plant of the backcross (*Gossypium armourianum* × *G. thurberi* F_1) × *G. armourianum* (pl. 1, *D*) is intermediate between that of the F_1 hybrid and that of *G. armourianum*. The mesophyll approaches that of *G. armourianum* in being more compact and having a greater number of cell layers than that of *G. armourianum* × *G. thurberi* F_1, but it is neither quite so compact nor quite so thick as that of *G. armourianum*.

The structure of the mesophyll of the reciprocal backcross (*Gossypium armourianum* × *G. thurberi* F_1) × *G. thurberi* (pl. 1, *E*) is intermediate between that of *G. armourianum* × *G. thurberi* F_1 and that of *G. thurberi*. In this backcross plant the mesophyll is slightly thicker than that of *G. thurberi* because of a somewhat greater development of spongy parenchyma. The spongy parenchyma of the backcross plant contains many cells that are considerably more elongate than in the corresponding tissue of *G. thurberi:* but the spongy parenchyma is little if any more compact in the backcross than in *G. thurberi*, since it contains numerous large intercellular spaces.

Second-Generation Hybrids

An examination of leaves representing 29 plants from selfed seed of W8 and 25 plants from selfed seed of W9 showed that the mesophyll of F_2 plants (pl. 1, *F, G, H, I, J, K*) varies considerably. The F_2 population comprised plants in which the mesophyll closely resembled, respectively, that of *Gossypium thurberi* (pl. 1, *F*, as compared with pl. 1, *B*), *G. armourianum* × *G. thurberi* F_1 (pl. 1, *G*, as compared with pl. 1, *C*), the backcross (*G. armourianum* × *G. thurberi* F_1) × *G. armourianum* (pl. 1, *H*, as compared with pl. 1, *D*), and the backcross (*G. armourianum* × *G. thurberi* F_1) × *G. thurberi* (pl. 1, *I*, as compared with pl. 1, *E*). In other F_2 plants the mesophyll structure differed from all of the foregoing. Noteworthy among such additional types of mesophyll structure are those shown in plate 1, *J* and *K*. Plate 1, *J*, represents a cross section of a relatively thin leaf in which the chlorenchyma is composed of four layers of palisade cells; plate 1, *K*, represents a relatively thick leaf in which spongy parenchyma is present.

If the F_2 population is considered as a whole, it may be observed that the mesophyll contains a considerably larger proportion of palisade



Cross sections of petioles of *Gossypium*, taken at approximately the midpoint, × 33: *A*, *G. armourianum*; *B*, *G. armourianum* × *G. thurberi*, F_1, plant W9; *C*, *G. thurberi*; *D*, the backcross (*G. armourianum* × *G. thurberi*, F_1) × *G. armourianum*, F_1; *E*, the backcross (*G. armourianum* × *G. thurberi*, F_1) × *G. thurberi*, F_1; *F*, W9, F_2, plant 17; *G*, W9, F_2, plant 4; *H*, W8, F_2, plant 6; *I*, W8, F_2, plant 21; *J*, W9, F_2, plant 25; *K*, W9, F_2, plant 12; *L*, W9, F_2, plant 18; *M*, W9, F_2, plant 11; *N*, W8, F_2, plant 19; *O*, W9, F_2, plant 20.

tissue than occurs in the mesophyll of the leaves of *Gossypium thurberi*. Typical loose spongy parenchyma, similar to that adjoining the lower epidermis in the leaves of *G. thurberi*, is present in the mesophyll of but 13 of the 54 F_2 plants examined. In the remaining 41 F_2 plants the mesophyll is in all cases more compact than that of *G. thurberi*, and in many instances denser than that of *G. armourianum* × *G. thurberi* F_1. Typical palisade parenchyma adjoins both the upper and lower epidermis of the leaves of 23 of these plants.

STRUCTURE OF THE PETIOLE

The Parent Species

Cross sections taken at approximately the midpoint of the petiole of *Gossypium armourianum* are oval or round in outline and have a continuous ring of fibrovascular tissue (pl. 2, *A*). The diameter of petioles of *G. armourianum* is among the smallest diameters occurring in the genus *Gossypium*.

Analogous sections of the petioles of *G. thurberi* (pl. 2, *C*) are commonly quadrangular in outline and of considerably larger diameter than the petioles of *G. armourianum*. Fibrovascular tissue in cross sections taken at the midpoint of petioles of *G. thurberi* occurs chiefly in four conspicuous bundles underlying the corners of the petiole. Additional fibrovascular tissue is often developed to a slight extent between the four major bundles, but it never occurs in sufficient amount to produce a continuous fibrovascular cylinder of fairly uniform width like that in *G. armourianum*.

First-Generation Hybrids and Backcrosses

Cross sections of the petioles of *Gossypium armourianum* × *G. thurberi* F_1 (pl. 2, *B*), though intermediate in size between those of the parents, bear a somewhat closer structural resemblance to *G. armourianum* than to *G. thurberi*. Their outline tends to be more angular than that of the petioles of *G. armourianum* but is distinctly rounder than that of the petioles of *G. thurberi*. The fibrovascular tissue occurs in a ring, which, except for somewhat larger size and a slightly less uniform distribution of vessels, resembles the fibrovascular ring in analogous sections of petioles of *G. armourianum*.

The size of cross sections taken at approximately the midpoint of the petiole of the backcross (*Gossypium armourianum* × *G. thurberi*, F_1) × *G. armourianum* (pl. 2, *D*) is nearly the same as that of analogous sections of the petioles of *G. armourianum* × *G. thurberi* F_1, with an approach to the *G. armourianum* parent in their smaller fibrovascular ring and slightly more rounded outline. Likewise, sections taken at about the midpoint of the petioles of the reciprocal backcross (*G. armourianum* × *G. thurberi* F_1) × *G. thurberi* (pl. 2, *E*) are of a size approximating that of analogous sections of petioles of *G. armourianum* × *G. thurberi* F_1 rather than of *G. thurberi*. An approach to the latter species is shown, however, by a more angular outline than in F_1 and by the uneven distribution of vessels within the fibrovascular ring, suggestive of four contiguous bundles rather than a continuous cylinder.

Second-Generation Hybrids

Individual plants in the F_2 population of *Gossypium armourianum* × *G. thurberi* differ greatly in the size, angularity, and distribution of fibrovascular tissue in their petioles. Comparable cross sections of the

petioles of these F_2 plants (pl. 2, *F–O*) range in size from about that of cross sections of petioles of *G. armourianum* (pl. 2, *G, O*, as compared with pl. 2, *A*) to nearly that of cross sections of petioles of *G. thurberi* (pl. 2, *I, M*, as compared with pl. 2, *C*), but mostly approximate in size the cross sections of *G. armourianum* × *G. thurberi* F_1 (pl. 2, *B*). The diameter of sections of petioles of the F_2 plants shows no correlation with their outline nor with the arrangement of fibrovascular tissue within the petiole. Sections of either relatively small diameter (pl. 2, *G, O*) or relatively large diameter (pl. 2, *I, M*) may be rounded (pl. 2, *M, O*) or somewhat angular (pl. 2, *G, I*) and may have their fibrovascular tissue disposed in a ring (pl. 2, *G, M*) or in several distinct bundles (pl. 2, *F, I*). Cross sections of the petioles of some of the F_2 plants rather closely resemble those of *G. armourianum* (pl. 2, *O*, as compared with pl. 2, *A*), *G. thurberi* (pl. 2, *I*, as compared with pl. 2, *C*) or *G. armourianum* × *G. thurberi* F_1 (pl. 2, *H*, as compared with pl. 2, *B*). Others show arrangements of fibrovascular tissue differing from those found in either parent species, or in F_1 (pl. 2, *K, L, N*).

The arrangement of vascular tissue in the petioles of the F_2 population as a whole shows greater resemblance to *Gossypium armourianum* or to *G. armourianum* × *G. thurberi* F_1 than to *G. thurberi*. A tendency to dominance of characters of *G. armourianum*, rather than of *G. thurberi*, appears therefore in the anatomy as well as in the gross morphology of the F_2 plants. Four comparatively large and widely spaced fibrovascular bundles, such as characterize the cross sections taken at approximately the midpoint of the petioles of *G. thurberi*, are present in analogous sections of petioles of only 12 of the 54 F_2 plants examined. Moreover, in the sections of petioles from these 12 plants the fibrovascular bundles are closer to one another than they are in comparable sections of the petioles of *G. thurberi*. The F_2 population, as a whole, also shows greater resemblance to *G. armourianum* or to *G. armourianum* × *G. thurberi* F_1 than to *G. thurberi* in that the petioles are much more frequently roundish or slightly angular than distinctly quadrangular.

SIGNIFICANCE OF THE ANATOMICAL FINDINGS

The number of plants in the F_2 population was too small to furnish a reliable basis for conclusions as to the inheritance ratios of characters in hybrids between wild American cottons, but is large enough to be indicative of the trends in structural features of the leaves. Such data are of interest, not only because of the bearing they may have on the origin of certain characteristics of cultivated American cottons, but also, in view of the relation between the morphology and the physiology of leaves, because of their possible significance to the plant breeder.

Relationships between morphological and physiological characteristics of the lamina have received much attention from ecologists. Consequently the influence of various environmental factors, particularly illumination, on structure and function of the epidermis and mesophyll of the leaf has been well established for many plants. Despite the recognized variability of leaves of a given species when grown under varying conditions, it is not uncommon for different species of the same genus to show structural differences that are sufficiently constant to be employed for taxonomic purposes. As may be seen from the literature cited by Halma (*1*), morphological

differences in the mesophyll of different species of the same genus have been correlated with physiological differences between such species. It has been found that in some genera the growth rate and productiveness of the species show a close positive correlation with the relative development of palisade tissue in the mesophyll.

The existence of correlations between the physiological behavior of the several species of *Gossypium* and differences in the development of palisade tissue in their leaves has not been determined. Consequently, the relationships established for other genera can only be regarded as suggestive in the case of *Gossypium*. However, since in other genera the desirability, from the practical standpoint, of obtaining plants with a relatively large proportion of palisade tissue is apparent, the possible existence in *Gossypium* of a correlation between desired physiological characteristics and abundant palisade development should not be overlooked. In view of this possibility, it is interesting that 41 of the 54 F_2 plants of *G. armourianum* × *G. thurberi* have a larger proportion of palisade tissue in their mesophyll than occurs in *G. thurberi*, the parental species possessing typical spongy parenchyma. The fact that there is a much higher proportion of palisade tissue in the mesophyll of the leaves of *G. armourianum* Kearney and *G. harknessii* Brandeg. than in the leaves of *G. klotzschianum* Anderss., *G. davidsonii* Kellogg, *G. thurberi* Tod., and *Erioxylum aridum* Rose and Standl. (*G. aridum* Skovsted) suggests that of these American wild cottons the two first-named species may be the most useful for breeding purposes.

Regardless of whether or not a correlation between palisade development and desirable physiological characteristics is found to exist in *Gossypium*, the tendency of hybrids between *G. armourianum*, a species characterized by centric leaves, and *G. thurberi*, a species with bifacial leaves, to have a more compact mesophyll structure than that occurring in the parent with bifacial leaves is of significance from the viewpoint of phylogeny. Since the leaves of the American cultivated cottons commonly have a layer of spongy parenchyma adjoining their lower epidermis (*9*), it may be inferred that at least one of the species involved in the origin of the American cultivated cottons possessed a mesophyll with typical spongy parenchyma. Among the American wild cottons a mesophyll of this type is known to occur in *G. davidsonii*, *G. klotzschianum*, and *Erioxylum aridum* as well as in *G. thurberi*. On the other hand, the mesophyll of *G. harknessii* resembles that of *G. armourianum*. Nothing is known concerning the mesophyll structure of the Mexican wild species *G. trilobum* (DC) Kearney (*Ingenhouzia triloba* DC) and *G. gossypioides* (Ulbr.) Standl. (*Selera gossypioides* Ulbr.) or of the Peruvian wild species *G. raimondii* Ulbr.

The structure of the petiole has often been profitably employed for taxonomic purposes, especially as an aid in specific diagnosis, but efforts to correlate petiole structure and the physiological characteristics of leaves are apparently wanting. Hence a comparison of analogous sections of petioles of hybrids between *Gossypium armourianum* and *G. thurberi* is of value at present chiefly from the taxonomic standpoint. As indicated above, petioles that are roundish or only slightly angular predominate in hybrids between *G. armourianum*, a species with petioles that are roundish in cross section, and *G. thurberi*, a species with petioles that are distinctly quadrangular in cross section. There is also a marked tendency for the fibrovascular tissue

in the petioles of these hybrids to occur more frequently in a closed cylinder, as in *G. armourianum*, than in separate fibrovascular bundles, as in *G. thurberi*.

At present too little is known concerning the structure of petioles of the cultivated American cottons to afford a basis of speculation as to what the foregoing indications of dominant and recessive characters may suggest as to the type or types of petioles possessed by the species involved in the origin of the cultivated American cottons. However, in view of the reported similar cytological behavior of such morphologically distinct species as *G. armourianum* and *G. thurberi* (*10*), it would seem that in seeking to establish the phylogenetic origin of the cultivated American cottons their morphological characteristics should be considered in conjunction with the dominance relations of morphological characters in hybrids involving species with 13 chromosomes.

SUMMARY

Two wild, lintless, 13-chromosome species of cotton, *Gossypium armourianum* and *G. thurberi*, both native in North America, were crossed in an experiment at Riverside, Calif.

The resulting hybrid proved to be much more fertile than any other hybrid that has been obtained by crossing two truly wild species of *Gossypium*. F_2 populations, totaling 64 individuals, were grown in 1937 and 1938.

Interspecific hybrids in *Gossypium* are likely to prove important in contributing to a better understanding of the phylogeny and taxonomy of the genus; and study of their morphology and cytological behavior may help to solve the problem of the origin of the cultivated American cottons with 26 chromosomes. There is also a possibility that they may be utilized eventually in breeding cottons superior in resistance to drought, diseases, and insect pests.

The first part of this paper deals with the gross morphological characters of the parent species and their inheritance in the hybrid.

Some of the more conspicuous contrasting characters of *Gossypium armourianum* and *G. thurberi*, and their expression in the first generation of the hybrid (F_1), are stated. The hybrid resembles the *G. armourianum* parent in six of these characters and the *G. thurberi* parent in two characters. In six characters, the hybrid is more or less intermediate between the parent species.

The classifications of the second-generation (F_2) population in respect to these characters are also presented. There is strong indication of simple Mendelian or monohybrid segregation in only one character, shape of cross section of the twigs. There is close approach to a 1:2:1 ratio in this instance.

The tendency to dominance of a greater number of genes from *Gossypium armourianum* than from *G. thurberi* is evident in F_2, as well as in F_1.

A reversal of the dominance tendencies of the characters petal spot and pollen color, as shown in intraspecific crosses and in hybrids between different species of cultivated American cottons, is indicated in *Gossypium armourianum* × *G. thurberi* F_2. The population is much too small, however, to warrant a definite conclusion on this point.

No detailed comparison of the anatomical characters of an interspecific hybrid in *Gossypium* with that of its parents appears to have

been published previously. The anatomical features of the mesophyll and the petiole alone are considered in this paper.

As in the gross morphological features, the hybrid resembles *Gossypium armourianum* more closely than *G. thurberi* in its histological characters.

The compact mesophyll and closed fibrovascular cylinder of the petiole of *Gossypium armourianum* are partly dominant to the relatively loose mesophyll and separate fibrovascular bundles of *G. thurberi*.

Consideration is given to the probable physiological advantage of a compact mesophyll with highly developed palisade tissue, such as characterizes *Gossypium armourianum*, and to the possible significance, in plant breeding, of leaf structure of this type.

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SOFT ROT OF PUMPKIN AND WATERMELON FRUITS CAUSED BY PYTHIUM ULTIMUM[1]

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INTRODUCTION

In connection with studies of a bacterial soft rot of Zucchini pumpkin fruits (*Cucurbita pepo* L. var. *condensa* Bailey), briefly described in a preliminary note by Ark and Tompkins (*2*)[3], a crate of field-infected fruits was shipped from Encinitas in southern California to Berkeley, Calif., in January 1936. Although some of these fruits upon examination showed bacterial soft rot, most of them were obviously involved in a fungus type of decay as revealed externally by soft, water-soaked areas partly covered with white mycelium. The causal fungus was identified as *Pythium ultimum* Trow and its pathogenicity proved in inoculation tests. Later the disease was found on Mammoth Summer Crookneck pumpkin fruits at Salinas and on Klondyke watermelon fruits (*Citrullus vulgaris* Schrad.) obtained in a Berkeley market. This paper deals with symptoms of the disease, the causal organism, and host range.

REVIEW OF LITERATURE

Species of *Pythium* have frequently been recorded as pathogens of cucurbit fruits.

McRae (*28*) reported from India that strains of *Pythium* were associated with decay of *Luffa acutangula* Roxb., *L. aegyptiaca* Mill., *Trichosanthes anguina* L., and *Lagenaria vulgaris* Ser. Parisi (*31*) noted the occurrence of *P. debaryanum* on fruits of chayote (*Sechium edule* Swartz). Blossom-end decay of watermelons, caused by a fungus resembling *Pythium artotrogus* DeBary, was described by Drechsler (*6*) in 1923. He also found a fruit decay in the field associated with *P. debaryanum*, and described a disease of cucumber (*Cucumis sativus* L.) to which he gave the name cottony leak (*7*).

In 1928, Mitra and Subramaniam (*30*) also reported a fruit rot of various cultivated cucurbits in India, caused by *Pythium aphanidermatum* (Eds.) Fitz. Infection was observed on cucumber, *Cucumis melo* L. var. *momordica*, white-flowered gourd (*Lagenaria leucantha* Rusby), *Luffa acutangula*, *L. aegyptiaca*, balsam-pear (*Momordica charantia* L.), serpent or snakegourd (*Trichosanthes anguina*), and *T. dioica*. The symptoms were in close agreement with those described by Drechsler for cucumber (*7*). The disease occurred in

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[3] Italic numbers in parentheses refer to Literature Cited, p. 473.

the field during and after rains. Fruits on or near the soil level were readily attacked, and the disease was also observed to spread in storage.

Pythium myriotylum Drechsler, the cause of a decay of cucumber and watermelon fruits in the Southeastern States, was considered by Drechsler (*9*) to be one of the most destructive members of the genus. It is favored by high humidity and usually occurs in southern latitudes. Other species capable of rotting watermelons included *P. periplocum* Drechsler and *P. acanthicum* Drechsler. *P. debaryanum* was considered by Walker and Weber (*44*) to be one of the causes of blossom end rot of watermelon fruits in Florida. The disease is more commonly found on melons about one-fourth grown. Kheswalla (*24*) called attention to a rot of watermelons in Baluchistan caused by *P. aphanidermatum*.

TABLE 1.—*Plants reported susceptible to Pythium ultimum by various investigators*

Host	Locality	Investigator
Apium graveolens L.	United States	Alexander, Young, and Kiger (*1*).
Arachis hypogea L.	Union of South Africa	Wager (*40, 41*).
Beta vulgaris L.	United States	Poole (*34, pp. 807–814*).
B. vulgaris var. *crassa* Alef.	Canada	Jones (*23*).
B. vulgaris var. *macrorhiza* Stev.	do	Do.
	United States	Alexander, Young, and Kiger (*1*).
Brassica oleracea L.	England	Trow (*39*).
	United States	Drechsler (*8*).
		Harter and Whitney (*15*).
		Walker (*43*).
	Union of South America	Wager (*41*).
B. rapa L.	United States	Poole (*34*).
Callistephus chinensis Nees.	Union of South Africa	Wager (*40, 41, 42*)
Carica papaya L.	do	Wager (*40, 41*).
Cupressus sp.	Rhodesia	Hopkins (*17*).
Dahlia sp.	Union of South Africa	Wager (*41*).
Daucus carota L.	Canada	Jones (*23*).
Dioscorea batatas Decne.	France	Labrousse (*25*).
Gossypium sp.	United States	Arndt (*3*).
Ipomoea batatas (L.) Lam.	Union of South Africa	Wager (*40, 41*).
	United States	Drechsler (*10*).
		Harter and Whitney (*15, 16*).
		Poole (*33, 34*).
Lactuca sativa L.	do	Alexander, Young, and Kiger (*1*).
Lepidium sativum L.	England	Trow (*39*).
Lycopersicum esculentum Mill.	United States	Alexander, Young, and Kiger (*1*).
		Horsfall (*18*).
	New Zealand	Wilson and Tilford (*45*).
Medicago sativa L.	United States	Brien and Chamberlain (*4*).
Nasturtium sp.	Union of South Africa	Buchholtz and Meredith (*5*).
Nicotiana tabacum L.	United States	Wager (*41*).
Papaver nudicaule L.	Union of South Africa	Alexander, Young, and Kiger (*1*).
Phaseolus vulgaris L.	do	Wager (*40, 41*).
	United States	Wager (*41*).
		Harter and Whitney (*15, 16*).
		Poole (*34*).
Picea engelmannii Engelm.	do	Rathbun-Gravatt (*35*).
Pinus banksiana Lamb.	do	Do.
P. resinosa Ait.	do	Do.
Pisum sativum L.	Union of South Africa	Wager (*41*).
Raphanus sativus L.	United States	Poole (*34*).
Rheum rhaponticum L.	Union of South Africa	Wager (*40, 41*)
Solanum tuberosum L.	England	Trow (*39*).
	United States	Poole (*34*).
	Canada	Conners.[1]
		Jones (*23*).
	France	Dufrénoy (*11*).
		Foex (*13*).
		Foex, Dufrénoy, and Labrousse (*14*).
		Labrousse (*25, 26, 27*).
Spinacea oleracea L.	United States	Pirone et al. (*32*).
	Germany	Fabel (*12*).
Striga lutea	Union of South Africa	Wager (*40*).

[1] CONNERS, I. L. THIRTEENTH ANNUAL REPORT OF THE CANADIAN PLANT DISEASE SURVEY 1933. Canada Dept. Agr., Expt. Farms Branch 128 pp. 1934. [Mimeographed.]

Hosts reported susceptible to *Pythium ultimum* are presented in table 1. Numerous other plants are reported as susceptible when grown in naturally infested soil inhabited by other soil fungi as well as by *Pythium ultimum*; for this reason these hosts are not listed. These hosts may be found by referring to the recent contributions of Horsfall (*18*, *19*, *20*, *21*), Horsfall, Newhall, and Guterman (*22*) and Pirone, Newhall, Stuart, Horsfall, and Harrison (*32*) on the efficacy of various methods for the control of damping-off.

SYMPTOMS OF THE DISEASE

If in contact with or near the surface of wet soil, either during the rainy season or immediately after irrigation, Zucchini and Mammoth Summer Crookneck pumpkin fruits, irrespective of age or size, are susceptible to infection. The disease is favored by cool weather. Infection may occur on any part of the fruit but is found most commonly at either the stem or blossom end. The first symptom observed on naturally or artificially infected fruits consists of one or several slightly sunken, soft, water-soaked lesions of irregular size and shape. Under favorable conditions, there is a rapid enlargement of the lesions, accompanied by a marked sinking and softening of the underlying tissues. In advanced stages, the lesions are frequently covered with a dense, closely adpressed, white mycelium which is in marked contrast to the luxuriant, cottony growth of mycelium produced on cucumber fruits by *Pythium aphanidermatum* (*7*). The inherent firmness characteristic of healthy fruits is destroyed soon after infection; invaded fruits break open when handled, exposing the odorless, water-soaked, decaying tissues. Fruits may be completely rotted within 6 to 10 days after the appearance of the first symptoms of infection.

The conditions which predispose watermelons to infection under field or market conditions were not investigated. The symptoms on this host closely correspond with those on pumpkin fruits except that the lesions are army brown (*36*)[4] in color. Large quantities of free liquid are released when badly decayed fruits are broken in handling.

THE CAUSAL FUNGUS, PYTHIUM ULTIMUM

The fungus was readily obtained in pure culture by the usual tissue-planting technique (*29*) from 30 diseased pumpkins and 6 watermelon fruits.

Freehand cross and longitudinal sections of artificially infected tissues were stained with fast green and Magdala red. The fungus was both intercellular and intracellular.

MORPHOLOGY

Cultures grown on corn meal, oatmeal, and potato-dextrose agar developed a copious amount of white cottony aerial mycelium, filling the space between the slope and the wall of the tube after 4 days at room temperature. Cultures on steamed corn meal produced a profuse aerial mycelium, filling the basal portion of the Erlenmeyer flasks after 7 days. Hyphae were cylindrical, smooth, and nonseptate when young, measuring 1.7 μ to 6.2 μ in diameter, average 3.4 μ, and becoming septate with age.

[4] Color determinations were made with the aid of Ridgway's manual (*36*).

Sporangia were present on the aerial growth in all cultures, being most numerous on potato-dextrose agar, moderately abundant on corn meal and oatmeal, and rare on steamed corn meal. Intramatrical sporangia were often produced, though never abundantly. Sporangia were spherical when acrogenous, measuring 14.5μ to 28.3μ in diameter, average 20.4μ (fig. 1, *D*). Occasional intercalary bodies were found which varied in size from 15.7μ in width and 19.3μ in length to 23.3μ ×27.3μ, average 19.6μ×23.9μ.

Tufts of mycelium from potato-dextrose agar were placed in pea broth and allowed to grow at room temperature. Transfers were made 4 days later to sterile distilled water and Petri's solution. Sporangia were produced abundantly in both solutions. All attempts to induce zoospore formation were futile, germination always occurring by the production of one to 5 germ tubes.

To study the formation of oogonia, antheridia, and oospores, the fungus was grown in clear media. Agar squares containing the organism were cut from 2-day-old potato-dextrose agar plates and transferred to Petri dishes containing corn-meal decoction agar and plain water agar. Oogonia, antheridia, and oospores were produced freely in these media in 3 to 4 days. Similar bodies were formed when tufts of mycelium were transferred from pea broth to sterile distilled water and Petri's solution.

Of the isolates from diseased cucurbit fruits, only those from Klondyke watermelon and Mammoth Summer Crookneck pumpkin produced oogonia. The isolate from Zucchini pumpkin used in the inoculation studies has never produced oogonia, even though a variety of media and procedures has been tried. Oogonia were largely intramatrical, acrogenous, spherical with a smooth thin wall, and measured 17.3μ to 24.2μ in diameter, average 20.2μ (fig. 2, *A*). Antheridia were generally androgynous, usually one to an oogonium, though occasionally two were present, in which case one was always of diclinous origin. Typical antheridia arose immediately below the oogonium on the oogonial stalk (fig. 2, *B*, *C*, *D*). They were somewhat clavate, short, upcurved, the tip in narrow contact with the oogonial wall. At maturity the contents of the oogonium differentiated into a peripheral layer, the periplasm, the remaining portion becoming the oosphere. A penetration tube was produced by the antheridium, permitting its contents to pass into the oosphere (fig. 2, *C*, *D*). After fertilization the oosphere further differentiated to form the oospore.

When mature the oospore was spherical, smooth, and thick-walled, not filling the oogonial cavity (fig. 2, *F*, *F*). The contents were somewhat granular. A reserve globule was present; frequently associated with it was a subspherical refringent body. The oospores measured 15.7μ to 22.9μ in diameter, average 17.4μ, including the oospore wall, which was approximately 1.4μ thick.

In addition to the reproductive bodies mentioned that formed in clear media, numerous swollen hyphal elements forming complex aggregations were found in contact with the bottom of the Petri dish (fig. 1, *A*, *B*). The hyphae were two to five times larger in diameter, and had denser contents than the parent hypha. They closely resembled the filamentous sporangia frequently found in *Pythium*, or the falcate bodies observed in *Pythium splendens*. Bits of agar containing these complexes were placed in sterile distilled water and

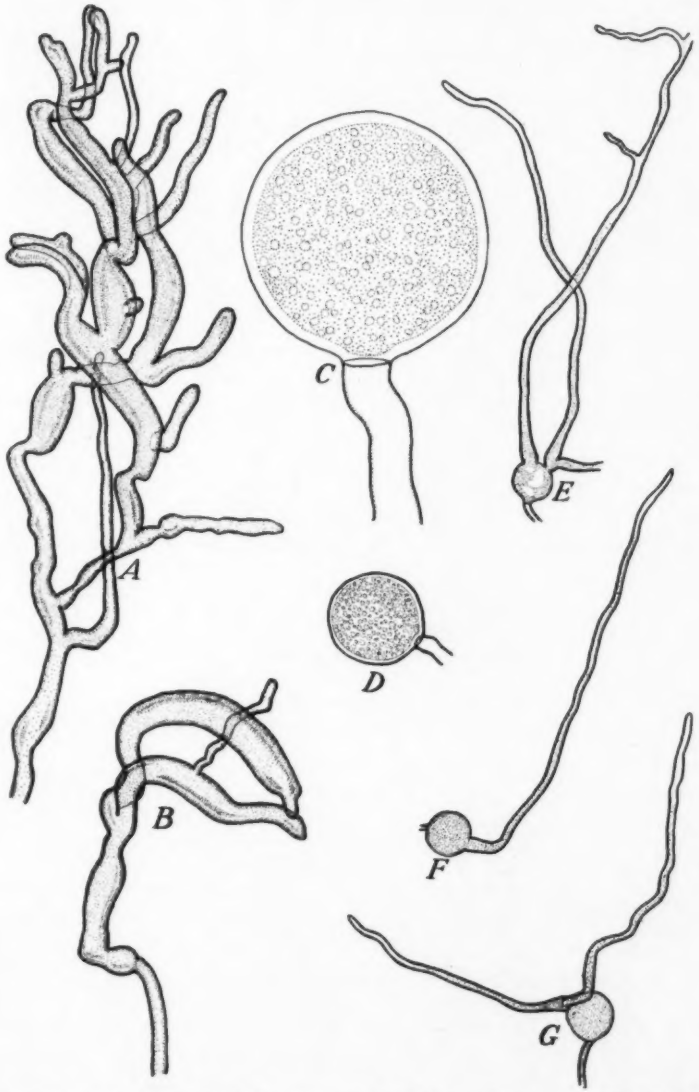

FIGURE 1.—*A*, *B*, Frustrated appressoria of *Pythium ultimum* (×675); *C*, reproductive body resembling oogonium (×1,250); *D*, terminal sporangium (×500); *E*, *F*, *G*, germination of oogonia by germ tubes (×250).

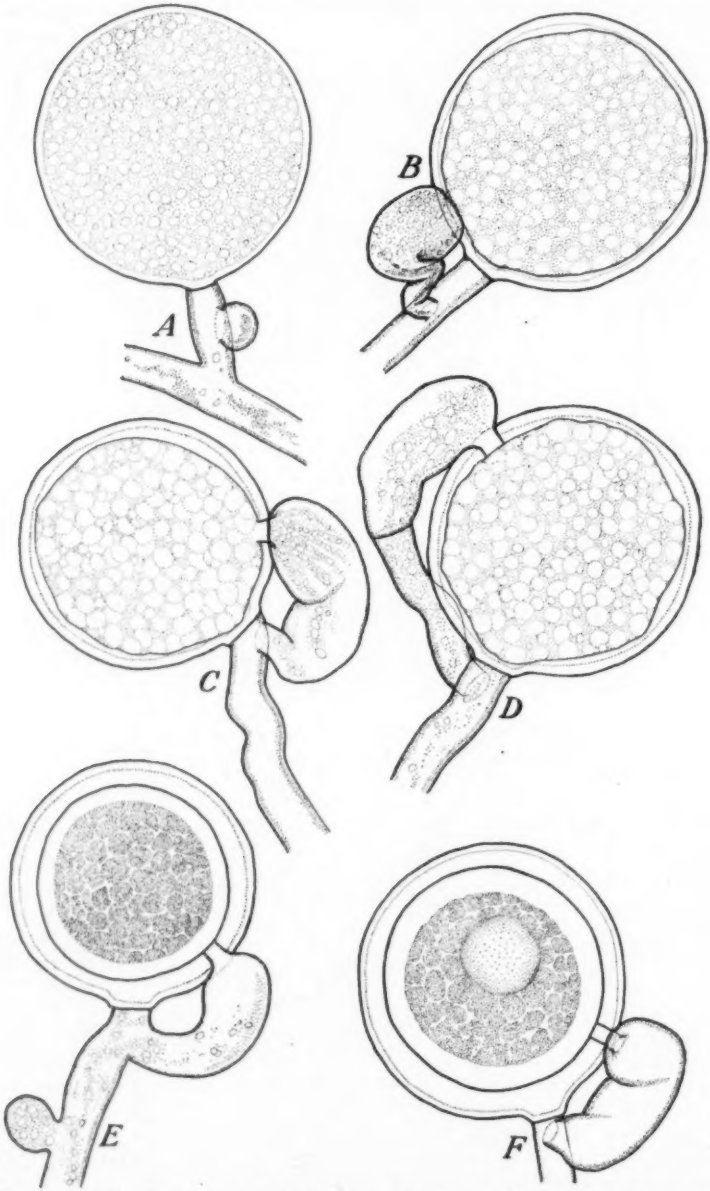

FIGURE 2.—Oospore formation in *Pythium ultimum*: *A*, Oogonium with antheridial element; *B*, antheridium in contact with oogonial wall; *C*, *D*, union of antheridial and oogonial contents; *E*, inmature oospore; *F*, mature oospore. *A*–*F* × 1,250.

Petri's solution in an attempt to stimulate zoospore development. Germination occurred by the production of normal hyphae arising from the tips of the enlarged threads; zoospores were never formed. Although these aggregations may be regarded as plasmatoögoses, the fact that they were produced only when the mycelium was in contact with the surface of the vessel, and never at random in the medium, leads the writers to consider them frustrated appressoria.

The causal organism corresponds quite closely with the fungus from *Lepidium sativum* described by Trow (*39*) as *Pythium ultimum*, and has been identified as that species by the writers. *P. ultimum* was originally described as a saprophyte; however, numerous reports in the literature refer to the species as an aggressive parasite of numerous hosts.

Of the species reported from cucurbits only *Pythium debaryanum* and *P. ultimum* produce spherical sporangia. Both have acrogenous spherical sporangia; the intercalary sporangia produced sparingly in *P. ultimum* are moderately abundant in *P. debaryanum*. Germination of the sporangia by germ tubes is common to both; however, zoospores are frequently produced by *P. debaryanum* but not by *P. ultimum*. The oogonia of *P. debaryanum* are smooth-walled and somewhat larger in diameter than those of *P. ultimum*. The intercalary oogonia frequently found in *P. debaryanum* are absent or extremely rare in *P. ultimum*. Several antheridia are produced in *P. debaryanum* which may be either diclinous or androgynous, but if the latter, they do not originate immediately below the oogonium as in *P. ultimum*. Mature oospores of both species lie free in the oogonial cavity. As indicated by Drechsler (*8*), a reserve globule is present in oospores of each species, though somewhat smaller in size in *P. ultimum*. A small refringent body is frequently associated with the globule; this is usually ellipsoidal in *P. debaryanum* and subspherical in *P. ultimum*. The oospore wall is approximately 1.0μ thick in *P. debaryanum* and 1.5μ in *P. ultimum*. Whether these two species should be maintained as separate entities is a moot question and should be further investigated.

A comparison of *Pythium ultimum* from fruits of *Citrullus vulgaris*, *Cucurbita pepo* var. *condensa*, roots of *Medicago sativa*, *Nicotiana tabacum* and *Spinacia oleracea* with Trow's original description and that reported by Wager (*40*) is presented in table 2.

It is apparent that the writers' isolates listed are identical and agree closely with the description of *Pythium ultimum* as set forth by Trow. Although two of the isolates from *Cucurbita pepo* failed to develop sexual organs it seems probable that they too should be referred to this species.

Reproductive bodies resembling oogonia were produced by the two cucurbit isolates which did not develop oospores (fig. 1, *C*). These were acrogenous, spherical, and thin-walled, and appeared intramatrically in clear media after 3 or 4 days. When a bit of agar containing the fungus was transferred to sterile distilled water the bodies germinated within an hour by the production of one to three germ tubes (fig. 1, *E*, *F*, *G*). These spherical bodies are considered oogonia rather than sporangia because similar structures were observed in cultures of isolates producing oogonia and oospores. The following procedure was used in this study.

TABLE 2.—*Spore measurements of Pythium ultimum isolations from Citrullus, Cucurbita, Medicago, Nicotiana, and Spinacia compared with measurements published by Trow and Wager*

Investigator and host	Oogonia			Oospores			Sporangia		
	Minimum	Maximum	Average	Minimum	Maximum	Average	Minimum	Maximum	Average
Trow (39)	μ	μ	μ	μ	μ	μ	μ	μ	μ
Levidium sativum	19.6	22.9	20.6	14.7	18.3	16.3	12.0	28.0	20.0
Wager (40):									
Arachis hypogea	15.8	27.0	22.5	13.5	20.3	18.3	18.0	27.0	23.0
Carica papaya	14.4	27.0	21.3	11.3	20.7	17.0	22.5	22.5	22.5
Ipomoea batatas	18.0	27.0	22.8	13.5	21.6	18.0	15.8	27.5	23.8
Papaver nudicaule	16.7	27.0	22.4	12.2	21.2	18.1	13.5	29.7	23.9
Rheum rhaponticum	15.8	25.7	22.1	13.5	22.1	17.8	15.8	31.5	24.2
Striga lutea	13.5	22.5	18.9	11.3	20.7	16.0	13.5	22.5	20.6
Baarn (host unknown)	15.6	25.4	21.3	13.7	22.5	17.5	15.3	26.8	21.2
Writers (Tompkins, et al.):									
Citrullus vulgaris	17.3	23.6	20.3	15.7	22.0	17.2	17.3	26.8	20.3
Cucurbita pepo	17.6	24.2	20.2	15.8	22.9	17.7	15.1	28.3	20.9
Cucurbita pepo							15.9	27.6	20.4
Cucurbita pepo							14.5	26.6	20.0
Medicago sativa	14.4	23.2	20.9	11.6	19.7	17.0	16.4	28.5	21.1
Nicotiana tabacum	16.8	24.1	20.6	14.1	20.8	17.1	15.8	26.9	20.7
Spinacia oleracea	14.8	24.2	21.1	11.7	21.9	17.6	16.2	27.3	21.3

Strips of agar with the mycelium of an oospore-producing isolate were removed from a 3-day-old plate culture. The strips were cut to include a well-developed parent hypha bearing terminal oogonia on short lateral branches. Strips were chosen in which oospores were present at the proximal end of the hypha, nearest the origin of growth, while at the young distal end there were only unfertilized oogonia with no differentiation of oospores. The agar blocks were placed in sterile distilled water and within a short time the spherical bodies in the distal region germinated, producing one to three germ tubes. Germination of oospores was not observed. In comparable agar sections selected but not removed from the culture dish, oospores developed in the distal bodies within 24 hours. There can be little doubt that the reproductive structures on the younger portion of the hypha were oogonia. In appearance and behavior they appeared identical with the terminal bodies on the short lateral branches that developed in the nonoospore-producing isolates.

It seems probable that the failure of certain isolates to develop oospores is the result of failure to form antheridia. Since there is no evidence that a heterothallic condition occurs in the species it is suggested that the monosexuality exhibited by these strains may be genetic in nature.

TEMPERATURE RELATIONS

The relation of temperature to growth of the mycelium was determined for isolates from fruits of Zucchini and Mammoth Summer Crookneck pumpkin, and from Klondyke watermelon. In addition to the organisms from cucurbit fruits, isolates from tobacco, alfalfa, and spinach roots and a culture from the Centraalbureau voor Schimmelcultures, Baarn, Netherlands, received as *Pythium ultimum*, were included. The culture tubes (2.1 by 20 cm) used and the procedure followed in this study were those previously described by Tompkins et al. (*37*). To each tube, provided with a glass dam at the open end,

15 cc of Difco corn-meal agar (pH 6.0) was added and allowed to cool at a uniform depth with the tube in a horizontal position, the dam preventing the escape of the melted agar.

These tubes were inoculated near the dam at the open end with small squares of potato-dextrose agar containing the mycelium of the fungus cut from 2-day-old Petri-dish cultures. Inoculated tubes were left at room temperature for 24 hours, the extent of growth at the close of that period being indicated by a wax-pencil mark on the tube: subsequent measurements were made from this point. Two tubes of each culture were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 4° to 40° C.

Growth occurred at all temperatures, all isolates behaving similarly. Perceptible growth occurred at 4° and 40° C. only after 72 hours exposure. Average growth over 24-hour periods for the various temperatures is presented in table 3.

The minimum temperature is approximately 4°, the optimum 25° to 28°, and the max mum 40° C.

TABLE 3.—*Mycelial growth of Pythium ultimum on Difco corn-meal agar at various temperatures*

Isolate No.	Average growth over 24-hour period at—												
	4° C.	7° C.	10° C.	13° C.	16° C.	19° C.	22° C.	25° C.	28° C.	31° C.	34° C.	37° C.	40° C.
	Mm	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*	*Mm*
1	0.08	1.8	4.1	7.0	11.3	19.7	27.7	31.1	31.4	29.0	22.1	10.3	0.15
2	.10	1.9	4.2	7.1	11.5	19.9	27.9	31.2	31.9	29.3	22.5	10.5	.20
3	.09	1.8	4.1	7.0	11.4	19.8	27.8	31.1	31.7	29.1	22.2	10.4	.19
4	.10	1.9	4.3	7.2	11.6	20.0	27.9	31.3	31.9	29.4	22.7	10.6	.21
5	.10	1.9	4.2	7.1	11.5	19.8	27.9	31.2	32.0	29.2	22.6	10.5	.20
6	.10	1.9	4.2	7.2	11.5	19.9	27.8	31.2	31.9	29.3	22.5	10.5	.20

These findings are in accord with other reports of temperature relations of *Pythium ultimum*. Jones (*23*) states that *P. ultimum* isolated from decayed tubers of potato was found to have a minumum temperature of approximately 4° C., an optimum of 28°, and a maximum of 40°. Likewise Wager (*40*) has found the minimum temperature of *P. ultimum* isolated from *Arachis hypogea*, *Carica papaya*, *Ipomoea batatas*, *Papaver nudicaule*, *Rheum rhaponticum*, and *Striga lutea* to be rather low, approximately 5°. The optimum was 30° with the exception of an isolate each from *C. papaya* and *S. lutea*, in which case the optimum was 35°. The maximum was 40° with the exception of the two isolates having a higher optimum. These latter cultures were found to have a correspondingly higher maximum temperature value. According to Pirone et al (*32*) the minimum temperature of *P. ultimum* isolated from spinach is somewhat lower than 9°, the optimum approximately 30°, the maximum 38°. Alexander, Young, and Kiger (*1*) report the optimum temperature of *P. ultimum* from tomato to be from 25° to 30°, the minimum about 10°. No maximum value is given.

Since temperature-growth relations are considered a specific feature, it seems quite probable that the cucurbit isolates are similar. The temperature relations are presented here in support of the identification of the cucurbit organisms as *Pythium ultimum*, and indicate physiological as well as morphological similarities.

PATHOGENICITY

The method of inoculation was essentially that described recently by Tompkins and Tucker (*38*). Inoculum was prepared by growing pure cultures of the three isolates of *Pythium ultimum* on malt-extract

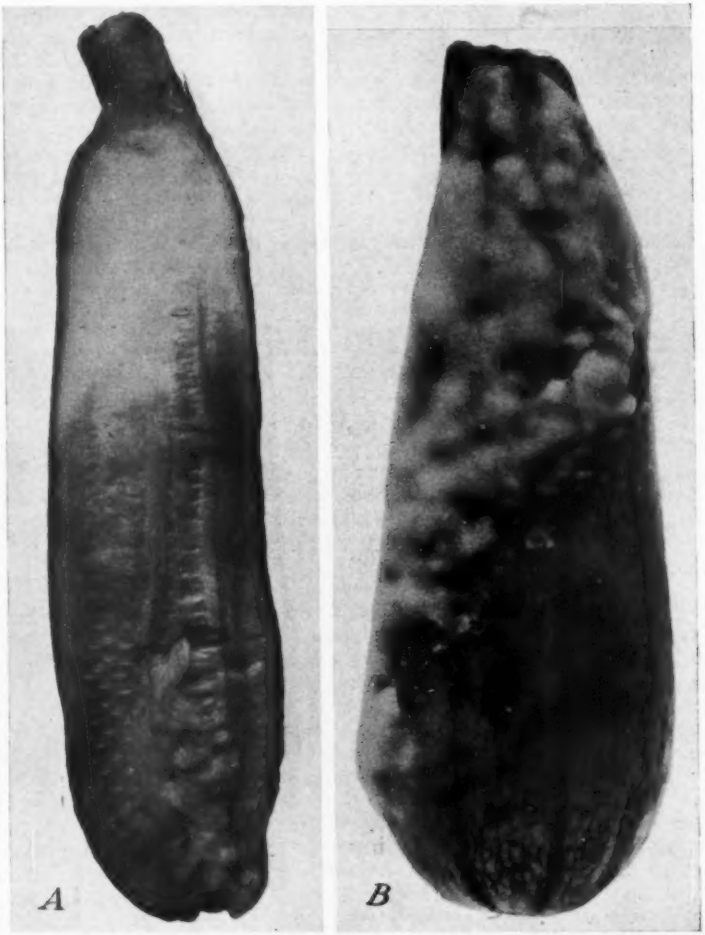

FIGURE 3.—Artificial infection of Zucchini pumpkin fruit: *A*, Longitudinal section of a diseased fruit, showing the soft, water-soaked tissues produced without wounding, 6 days after inoculation with a 3-day-old culture of *Pythium ultimum*. *B*, section from the same fruit, showing the type of aerial mycelium that develops in a moist chamber and a large water-soaked lesion free from mycelium. This fruit was completely rotted within 10 days.

agar in Petri dishes for 48 hours at room temperature. Healthy Zucchini and Mammoth Summer Crookneck pumpkin and watermelon fruits were washed in running tap water, rinsed in distilled water and dried. A small square of inoculum was placed on the uninjured

epidermis of the pumpkin fruits, and the fungus was inserted in small scalpel wounds through the rind of the watermelon fruits. The inoculum was kept moist with absorbent cotton under an inverted preparation dish. For controls, fruits were handled in the same manner except that sterile agar was substituted for the inoculum. All inoculations were made at room temperatures (20° to 23 ° C.).

Of 12 Zucchini pumpkin fruits inoculated, 3 were infected after 3 days as indicated by the soft, sunken, water-soaked lesions which measured approximately 7 cm in diameter and slightly more internally (fig. 3, *A*). After 4 days, four additional fruits were infected, with lesions averaging 10 cm in diameter. All the remaining fruits which showed infection after 5 to 7 days had lesions with an average diameter of 12 cm, except one which was completely rotted. These artificially induced lesions were identical in color and consistency with those on naturally infected fruits. The six noninoculated control fruits remained healthy. The fungus was reisolated from each infected fruit, and subsequently 10 healthy fruits were inoculated with the reisolated fungus. All inoculated fruits were diseased at the end of 5 days. Superficial growth of mycelium was not observed unless infected fruits were placed in moist chambers (fig. 3, *B*).

Comparable results were obtained in tests with Mammoth Summer Crookneck pumpkin fruits. Of nine fruits inoculated, eight were infected in 3 to 4 days. Of four Klondyke and four Cooper watermelons inoculated, all were infected within 6 days and totally decayed within 10 days. Inoculations with the reisolates from Mammoth Summer Crookneck pumpkin and watermelon fruits were successful.

Isolates from each of the three hosts proved pathogenic to the other two. However, infection of watermelon fruits with the two isolates from pumpkin fruits occurred only after wounding.

No infection occurred when cultures of the fungus on sterilized cracked wheat were introduced into autoclaved soil in 6-inch pots containing Zucchini and Mammoth Summer Crookneck pumpkin and watermelon seedlings; however, when the inoculum was placed in the soil immediately after seeding, damping-off occurred. About 90 percent of the plants were killed in the preemergence stage, and the remainder collapsed soon after emergence.

Isolations of *Pythium ultimum* from cucurbit fruits, roots of alfalfa, spinach, and tobacco, and the Baarn culture (host unknown) caused damping-off of tomato seedings; 50 tomato seeds were sown in infested soil in each of five 6-inch pots and survival counts were made 15 days later. The following results were obtained:

Source of isolate	*Seedlings surviving after 15 days*
Baarn (host unknown)	3
Citrullus vulgaris	10
Cucurbita pepo	2
Do	2
Do	2
Medicago sativa	3
Nicotiana tabacum	3
Spinacia oleracea	23
Check (not inoculated)	220

All isolates behaved similarly, including the nonoospore-producing isolates from cucurbit fruits. The damping-off symptoms were identical with those reported for *Pythium ultimum* on tomato and other seedlings by other investigators.

From these tests, it is concluded that *Pythium ultimum* is the cause of the fruit rot described here, and that it is an aggressive parasite capable of direct penetration of pumpkin but not of watermelon tissues.

EXPERIMENTAL HOST RANGE

Various hosts were inoculated by the no-wound technique described for the pathogenicity tests. Reisolations were made from all diseased specimens. The reisolate of the fungus from a particular host was then tested by inoculation into healthy specimens of that host.

The experimental host range of the three isolates of *Pythium ultimum*, as determined by artificial inoculations made simultaneously in the laboratory, is quite limited. Fruits of green and ripe tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Stone, bean (*Phaesolus vulgaris L.*), eggplant (*Solanum melongena* L. var. *esculentum* Nees), and field pumpkin (*Cucurbita pepo* L.) proved susceptible. Lesions which developed after 2 to 5 days were soft, water-soaked, and slightly sunken in appearance except those on eggplant which were warm sepia (*36*) in color.

No infection was obtained on unwounded fruits of apple (*Malus sylvestris* Mill.) var. Newtown Pippin, bell or green sweet pepper (*Capsicum annuum* L. var. *grossum* Sendt.), squash (*Cucurbita maxima* Duchesne) var. Banana, pumpkin (*C. pepo* var. *condensa*) var. Early White Bush Scallop and Pie, watermelon (*Citrullus vulgaris*), Casaba and Honeydew melons (*Cucumis melo* L. var. *inodorus* Naud.), Persian melon (*C. melo* var. *reticulatus* Naud.), and cucumber (*C. sativus*); on unwounded roots of turnip (*Brassica rapa* L.) var. Purple Top White Globe, rutabaga (*B. campestris* L. var. *napo-brassica* DC.), carrot (*Daucus carota* L. var. *sativa* DC.), and parsnip (*Pastinaca sativa* L.); and on unwounded potato tubers (*Solanum tuberosum* L.) var. Russet Burbank.

All fruits, roots, and tubers which had reacted negatively to inoculation without wounding, as noted in the preceding paragraph, were readily infected upon wounding. In addition, fruits of lemon (*Citrus limonia* Osbeck) and sweet orange (*C. sinensis* Osbeck), storage organs of Bermuda onion (*Allium cepa* L.) and sweetpotato proved susceptible when wounded. Soft, slightly sunken, water-soaked lesions appeared on fruits and roots within 3 days and on potato tubers in 4 days. Internally, infected tubers had a blackened cortex; the parenchyma was unchanged in color and soft in consistency.

SUMMARY

A soft rot of Zucchini and Mammoth Summer Crookneck pumpkin and watermelon fruits, prevalent in California, is described.

The disease occurs when fruits of any size or age are in contact with wet soil and is favored by cool weather.

Symptoms of the disease consist of soft, sunken, water-soaked lesions which quickly enlarge, causing the underlying tissues to collapse. Invaded fruits may be completely rotted within 6 to 10 days after infection occurs.

The causal organism has been identified as *Pythium ultimum* Trow.

Certain cucurbit isolates did not produce oospores, but were indistinguishable from normal cultures by growth or pathogenic characters.

Reproductive bodies resembling oogonia developed in cultures of nonoospore-producing isolates. It is suggested that the absence of oospore formation is the result of failure of the organism to develop antheridia.

The temperature relations of the organism were determined. The minimum was 4° C., optimum 25° to 28°, maximum 40°. All cucurbit isolates, including the two nonoospore-producing cultures, isolates from roots of alfalfa, tobacco, and spinach and a culture from an unknown host, had identical temperature relations.

Infection of healthy pumpkin and watermelon fruits was obtained by inoculation in the laboratory. However, wounding of watermelon fruits was necessary in order to induce decay.

All isolates caused damping-off of pumpkin, watermelon, and tomato seedlings during the preemergence and small-seedling stages of growth. Older plants were resistant.

Green and ripe tomatoes, eggplant, beans, and field pumpkin proved susceptible to infection, without wounding, under laboratory conditions. When wounded, the following hosts proved susceptible: Apple, bell pepper, squash, pumpkin (two varieties), watermelon, Casaba, Honeydew, and Persian melons, cucumber, turnip, rutabaga, carrot, parsnip, potato, lemon, sweet orange, onion, and sweetpotato.

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